INDIVIDUAL DISTINCTIVENESS, SHORT- AND LONG-TERM COMPARISONS, AND CONTEXT SPECIFIC RATES OF FLORIDA MANATEE VOCALIZATIONS

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ABSTRACT

In the 1980's, Thomas J. O'Shea recorded captive and wild Florida manatees (*Trichechus manatus latirostris*), and statistical analyses of various acoustic features of their calls indicated possible individual distinctiveness. To further test the hypothesis that manatee calls contain individually distinctive features and to test the hypotheses that various acoustic features of their calls are stable over short (1-3 year) and long (19+ year) time periods and that vocal rates vary depending on behavior, recordings of wild and captive manatees were made between November 2002 and January 2004. Recordings of 31 manatees (21 wild, 10 captive) were obtained and vocalizations from two additional manatees recorded only in the 1980's were analyzed, for a total of 33 individuals. Four of these manatees had been recorded both in the 1980's and in 2002-2004, and the vocalizations of these individuals were used for long-term comparisons. Cross-validated linear discriminant analyses using nine different parameters of manatee vocalizations determined that 1) when using only the most recent recordings, vocalizations were classified to the correct individual a greater percentage of time than expected by chance for 30 out of 33 animals; 2) an overall higher percentage of calls was correctly assigned to only one of four animals recorded in the 1980's when using all recordings versus just the most recent recordings; 3) when using only adult and calf vocalizations, calls were assigned correctly to both adults (82.2% of 506 vocalizations) and calves (79.5% of 503 vocalizations) a greater percentage of time than expected by chance; and 4) when using only calf vocalizations, calls were assigned correctly to both females (66.0% of 250 vocalizations) and males (58.1% of 253 vocalizations) a greater percentage of time than expected by chance. ANOVAs were performed on each of the nine parameters for individual manatees that were recorded: 1) over 19+ years, 2) when less than one year old and again between the ages of one and three, and 3) as adults in at least two separate

field seasons. For three individuals recorded over 19 years, 47% of tested parameters did not significantly change over that time period; all three individuals were calves in the 1980's. For the one individual recorded over 22 years, 33.3% of his parameters did not significantly change over that time period. For four individuals recorded when they were less than one year old and then again between the ages of one and three, 71% of tested parameters did not significantly change. For four individuals recorded as adults in at least two separate field seasons, 76% of tested parameters did not significantly change. These results indicate that manatee vocalizations are individually distinctive, and that there are age and sex differences in calls. These results also suggest that 1) many call parameters are apparently not stable from calf to adulthood in at least some individuals, 2) some calves, subadults, and adults show variable changes in parameters over short (1-3 year) time periods, and 3) some calves and adults show stability in all parameters over short time periods. Florida manatee vocalization rates were not affected by group size at Blue Spring State Park, Florida, although they were found to differ depending on behavior. When looking at call rates per minute averaged across group sizes, significantly higher vocalization rates occurred during social activity than during bottom resting, with boat, and play; mill had significantly higher rates than bottom rest and with boat; and surface rest had significantly higher rates than bottom rest. Overall, the hypothesis that Florida manatee vocalizations are stable over long time periods was not supported, although only one adult was tested. Conflicting evidence for short-term stability in both calf and adult calls was found, with the number and type of parameters that changed being highly variable. Results supported the hypotheses that Florida manatees have individually distinctive vocalizations and that vocal rates vary depending on behavior. These results add to a growing body of information on manatee behavior and communication, and could potentially contribute to manatee research and

conservation efforts by providing a means to document the presence of specific individuals, the presence of manatees from different age classes and sexes, or the occurrence of certain behaviors without having to conduct boat-based surveys.

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INTRODUCTION

Manatees and dugongs are marine mammals in the Order Sirenia, which includes two families—Dugongidae and Trichechidae. The dugong (*Dugong dugon*) is the only extant species of Family Dugongidae. Family Trichechidae includes three species—*Trichechus manatus* (the West Indian manatee), *Trichechus inunguis* (the Amazonian manatee), and *Trichechus senegalensis* (the West African manatee). There are two subspecies of the West Indian manatee: the Antillean manatee (*Trichechus manatus manatus*) and the Florida manatee (*Trichechus manatus latirostris*). It is this latter subspecies, the Florida manatee, which is the subject of this research.

Manatee Vocalizations

Much research has been done on manatee anatomy, morphology, physiology, behavior, and ecology (e.g., Packard *et al.* 1989, Reynolds and Odell 1991, O'Keefe 1993, Reynolds and Wilcox 1994, Koelsch 1997, Marshall *et al.* 1998a, Marshall *et al.* 1998b, Reep *et al.* 1998, Reynolds 1999, Ripple 1999, Rommel and Reynolds 2000, Rommel *et al.* 2001, Anderson 2002, Wright *et al.* 2002, Deutsch *et al.* 2003, Rommel and Caplan 2003); however, to date, little research has focused on sirenian (manatee or dugong) vocalizations. Schevill and Watkins (1965) were the first to record vocalizations of manatees (*T. manatus latirostris*). They reported that manatee sounds were short in duration (between 0.15 and 0.5 seconds), could be complex at the beginning and end (sometimes these parts of their calls contained "subharmonics," which are additional sounds in between the harmonics), and if the vocalizations had harmonics, then the

first harmonic[1] had a greater intensity than the fundamental (Schevill and Watkins 1965). Evans and Herald (1970) found the same to be true of a captive Amazonian manatee's (*T. inunguis*) calls. The fundamental frequencies of the vocalizations of the two species differed; Florida manatee vocalizations were between 2.5 and 5 kHz and Amazonian manatee vocalizations were between 6 and 8 kHz (Schevill and Watkins 1965, Evans and Herald 1970). O'Shea *et al.* (2000) recorded captive and wild Florida manatees and found that the fundamental frequencies of their calls usually ranged between 1.5 and 5 kHz, supporting the findings of Schevill and Watkins (1965). In addition, O'Shea *et al.* (2000) found that most calls consisted of single syllables (or notes) and had complex harmonics that extended up to 20 kHz. Recent work by Nowacek *et al*. (2003) largely supported these earlier findings. Nowacek *et al*. (2003) found that both subspecies of the West Indian manatee produced vocalizations that "were harmonic complexes with small frequency modulations at the beginning and end." The vocalizations were described as ranging from pure tones to broader-band sounds, and the loudest frequency occurred at either the first or second harmonic (Nowacek *et al*. 2003). The highest frequency of a harmonic reported from Florida manatees at Crystal River, Florida and from Antillean manatees in Belize was around 16 kHz, although spectrograms in the paper showed harmonics that extended to higher frequencies (Nowacek *et al*. 2003).

Hartman (1969) divided Florida manatee sounds into three basic categories—chirp-squeaks, squeals, and screams. Besides these three categories, manatee sounds have been

[1] Authors have been inconsistent in their references to fundamental frequencies and harmonics. Some authors refer to the lowest frequency of a vocalization as the first harmonic and use this interchangeably with the term fundamental frequency (Alicea-Pou 2001, Nowacek *et al*. 2003). Those same authors then consequently call the first multiple of the lowest frequency band the second harmonic. I will be calling the lowest frequency the fundamental frequency and the first multiple of the fundamental frequency the first harmonic (Figure 1), which is typical of most published descriptions.

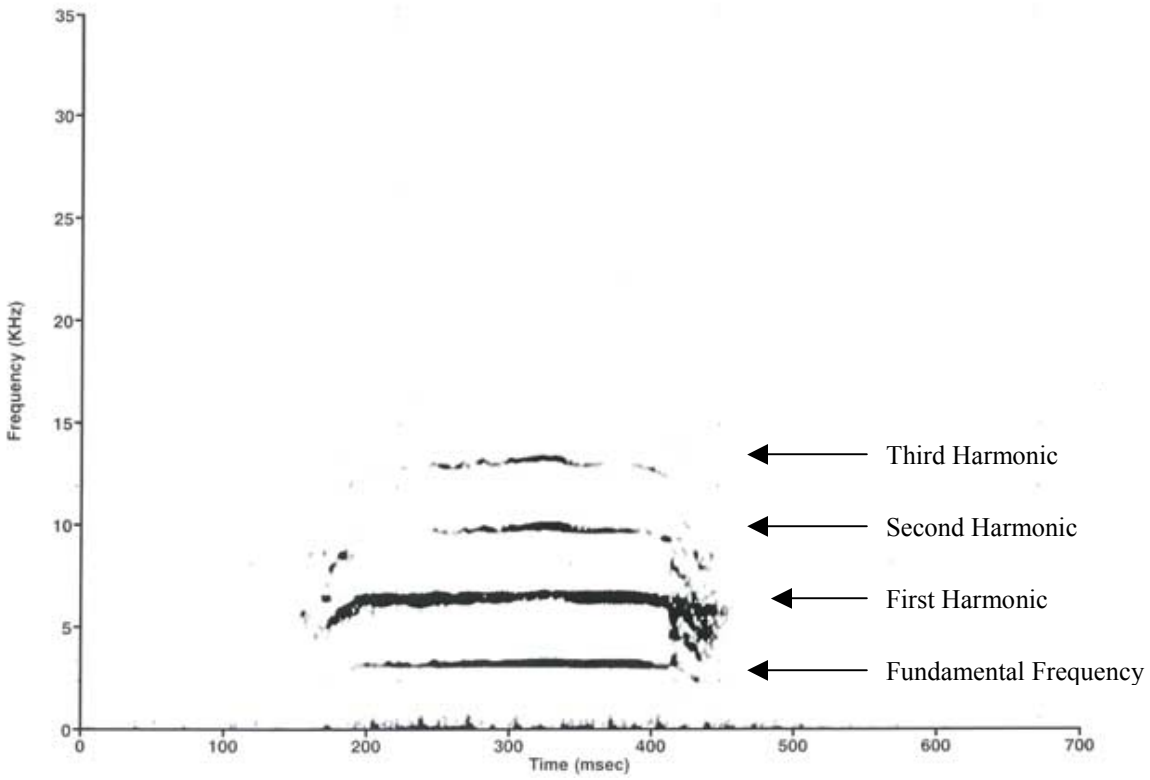

Figure 1. A manatee vocalization showing fundamental frequency and harmonic bands (sample rate: 80000 Hz; Hanning window; FFT size: 512; frequency resolution: 156 Hz). Frequency in kHz is on the y-axis and time in msec is on the x-axis.

classified as whistles, barks, trills, froglike calls, and rusty pump calls (Steel 1982, Wartzok and Ketten 1999, Reynolds and Odell 1991, Ripple 1999, Dudzinski *et al.* 2002). These classifications were based on how the vocalizations sounded to the human ear. Alicea-Pou (2001), who researched both subspecies of the West Indian manatee, classified manatee sounds by assigning each sound to one of five categories (or combinations of the five categories) based on both aural characteristics and visual inspection of spectrograms (Alicea-Pou 2001): 1) harmonic (multiple sound bands above the fundamental frequency); 2) formant-like (well-defined sound bands with no harmonics); 3) pulse (clear vertical spaces within the energy bands); 4) click (short duration and rapid onset—made by calves); and 5) noisy (no clear energy bands and sound energy covering a large range of frequencies). Within each category, he found calls that varied in different acoustic parameters (such as frequency, duration, modulation, etc.), but he classified these calls as variants of one of the five call types. Alicea-Pou (2001) also described irregularities that were found in between harmonic bands of some manatee vocalizations (called subharmonics), as described previously by Schevill and Watkins (1965).

Vocalizations in manatees are thought to be communicative in nature rather than navigational (Schevill and Watkins 1965, Evans and Herald 1970, Hartman 1969, O'Shea *et al.* 2000). Hartman (1969) stated that manatee vocalizations seemed to be related to emotional states. Bengtson and Fitzgerald (1985) found that call rates seemed to be associated with social factors, such as maintaining contact with others and identifying or greeting individuals, during which call rates increased. The elevated call rates heard when mother-calf pairs were present also suggest that manatee calls function in communication (Reynolds 1981, O'Shea *et al.* 2000, Alicea-Pou 2001), as did a playback experiment in which a mother reacted to the calls of her calf more than to calls of an unrelated calf (Sousa-Lima *et al.* 2002). Recently, Phillips *et al.* (2004)

played back Florida manatee vocalizations from Blue Spring, Florida to manatees at Homosassa Springs, Florida, and found that vocal rates increased when such calls were broadcast. Manatees also reacted physically to the broadcasts by aggregating around the speaker and becoming physically active (Phillips *et al.* 2004). In addition, the manatees (which were all female) formed a circle around the speaker, with their heads out, when a distressed calf's calls were broadcast (Phillips *et al.* 2004). Phillips *et al.* (2004) concluded that these behaviors in response to the broadcasted vocalizations suggest that Florida manatee calls are used in communication.

Individually Distinctive Vocalizations

Communicative signals in animals can be either shared call types or individually distinctive vocal signatures. A call type is shared when acoustic features of the call are similar among individuals of the same species. Shared calls may have "by-product distinctiveness" (Boughman and Moss 2003), which arises because of natural variation in individuals, including differences in body size and morphology of the vocal tract. In contrast, individually distinctive vocal signatures are stereotypic vocalizations that differ in acoustic features from those of other individuals belonging to the same species (Boughman and Moss 2003).

Individually distinctive signals (call types or vocal signatures) have been reported in species in which it is often necessary for a mother to locate her young, such as after a separation (Rasmuson and Barclay 1992, Sousa-Lima *et al.* 2002), and in species that live in fission-fusion societies where individuals join and leave groups while also maintaining long-term associations with other individuals (Smolker *et al.* 1993). Sousa-Lima *et al.* (2002) suggested that individually distinctive vocalizations are likely to occur in animals that invest a significant amount of time in parental care and have low reproductive rates. However, the occurrence of

individually distinctive vocalizations does not mean that these calls are used for individual recognition, as discussed further below. Animals in which individually distinctive vocalizations have been identified include big brown bats (*Eptesicus fuscus*; Rasmuson and Barclay 1992), evening bats (*Nycticeius humeralis*; Scherrer and Wilkinson 1993), Mexican free-tailed bats (*Tadarida brasiliensis mexicana*; Gelfand and McCracken 1986), timber wolves (*Canis lupus*; Tooze *et al.* 1990), Wied's black tufted-ear marmosets (*Callithrix kuhli*; Jorgensen and French 1998), common marmosets (*Callithrix jacchus*; Jones *et al.* 1993), squirrel monkeys (*Saimiri sciureus*; Symmes *et al.* 1979), fallow deer bucks (*Dama dama*; Reby *et al.* 1998), blue penguin chicks (*Eudyptula minor*; Nakagawa *et al.* 2001), and bottlenose dolphins (*Tursiops truncatus*; Tyack 1986, Caldwell *et al*. 1990). Anderson and Barclay (1995) suggested that dugongs (*Dugong dugon*) may also possess vocal signatures; the chirp-squeaks of dugongs showed individual variability in amplitude modulation and frequency.

Manatees

It is not known if individually distinctive vocalizations occur in manatees. Manatees are reasonable candidates for possessing individually distinctive vocalizations due to their long period of parental care and low reproductive rates (Sousa-Lima *et al.* 2002). Individually distinctive vocalizations could aid in maintaining contact between mothers and their calves. If, as suggested by Reynolds and Powell (2002), manatees live in fission-fusion societies, they also may use individually distinctive vocalizations to keep track of group members, especially in murky water or when widely separated (Reynolds 1981). Several studies have provided support for the idea that manatee vocalizations may be individually distinctive. Through statistical analyses of various acoustic features of the calls of Florida manatees, O'Shea *et al.* (2000) found

some evidence for individually distinctive vocalizations. Alicea-Pou (2001) could consistently identify certain manatees based on the characteristic sound quality and pitch of their vocalizations when making underwater observations. Alicea-Pou (2001) also found moderate variability among the vocalizations of four captive Antillean manatees when data were stratified by individual. He found that captive Antillean manatee vocalizations had "low intra-individual variability compared with the amount of inter-individual variability" and that "[e]ach individual [manatee] did not have a complex repertoire of distinctive and discrete call types" (Alicea-Pou 2001). He also reported that the vocalizations of a given individual manatee sounded alike to the human ear (Alicea-Pou 2001). Additionally, Sousa-Lima *et al.* (2002) found that captive Amazonian manatees (*T. inunguis*) produced "isolation calls" that differed in fundamental frequency and duration among individuals. Sousa-Lima and da Silva (2001) reported that two captive Amazonian manatees produced vocalizations with individually distinctive contours that were stable over a four-year period.

I tested the hypothesis that Florida manatee vocalizations have individually distinctive features. In addition to the intrinsic scientific interest of determining whether manatees produce individually distinctive vocalizations, this research could also be useful for manatee conservation efforts. For example, a remote acoustic monitoring system using hydrophones in different areas in Florida could potentially document the presence of specific individuals without having to conduct boat-based photo-identification surveys. Such acoustic monitoring could be especially useful in places where aerial, land, or boat-based surveys are difficult to perform due to poor visibility (Baptista and Gaunt 1997).

Individual Recognition

It is important to understand that although sounds can have individual variation, they may not necessarily be used for individual recognition. According to Beer (1970), there are three components necessary to documenting individual recognition: 1) a characterization of variability in calls among individuals, 2) observation of how vocalizations affect behavior, and 3) playback experiments to determine how animals react to sounds produced by different individuals. Evidence for individual variability in manatee calls was presented in the previous section. Some observational evidence in support of individual recognition also exists. Mothers are able to locate their calves in water with poor visibility when aggregating at warm water refuges (Alicea-Pou 2001). In addition, O'Shea *et al.* (2000) and Hartman (1979) found that mothers and their calves usually only responded to one another's calls, and Alicea-Pou (2001) found that other manatees responded to the vocalizations of only certain individuals.

As for the third component in documenting individual recognition, playback experiments found that a mother responded to her own calf's calls but not to an unrelated calf's calls (Sousa-Lima *et al.* 2002). Thus, preliminary data provide a strong foundation for pursuing the study of individual recognition in manatees. This work attempted to contribute to this line of research by examining individual variability in manatee calls as well as by observing manatee behavior concurrent with vocalizations.

Stability and Changes in Vocalizations Over Time

During Ontogeny

Vocalizations of some animals change over time, especially during ontogeny. Little brown bats (*Myotis lucifugus*) increased the frequencies and decreased the duration of their

echolocation calls during ontogeny (Moss *et al*. 1997). Pygmy marmosets (*Cebuella pygmaea*) modified their trill vocalizations as they aged from infancy to adulthood (Elowson *et al*. 1992); most marmosets decreased the frequencies and increased the duration of their trills.

Some animals may change one or more aspect(s) of their vocalizations during ontogeny while maintaining other aspects. For example, evening bats (*Nycticeius humeralis*) maintained stable contours of their isolation calls but decreased call duration and increased call frequency during the first two weeks of life (Scherrer and Wilkinson 1993). Mexican free-tailed bat pups (*Tadarida brasiliensis mexicana*) increased the frequencies of their isolation calls during ontogeny while maintaining stable contours (Gelfand and McCracken 1986).

During Adulthood

If vocalizations are to function effectively in long-term individual recognition, it follows that they should be stable throughout adulthood. Stability of individually distinctive vocalizations has been examined in several species. Female collared doves (*Streptopelia decaocto*), which are non-songbirds, showed stability in their perch-coos between the ages of two and 18 months (Ballintijn and ten Cate 1997). Squirrel monkey (*Saimiri sciureus*) isolation peeps (a shared call type that contains individually distinctive features) remained stable for up to four years (Symmes *et al.* 1979). Similarly, the individually distinctive "pyow" calls of one adult male blue monkey (*Cercopithecus mitis stuhlmanni*) remained stable over a 10-year period (Butynski *et al.* 1992).

As discussed previously, some species of animals may change one or more aspect(s) of their vocalizations over time while maintaining stability in other aspects. Common marmosets (*Callithrix jacchus*) maintained stable contours of their "phee" calls over a one-year period but

decreased the frequencies of their calls over that time period (Jones *et al.* 1993). Sayigh *et al.* (in prep.) investigated long-term changes (ranging from 5-25.5 years) in female bottlenose dolphin (*Tursiops truncatus*) signature whistles and found that most (15 out of 18) had stable contours over time. However, the highest frequency of their signature whistles increased until about age 20, at which point this parameter started to decrease; mean lowest frequency decreased steadily with age (Sayigh *et al.* in prep.). Wied's black tufted-ear marmosets (*Callithrix kuhli*) acoustically modified their "phee" calls over a period of two to three years by changing any number of eight parameters while others sometimes remained stable (including start and stop frequency, peak frequency, frequency range, call duration, syllable duration, inter-syllable duration, and number of syllables in a call; Jorgensen and French 1998). Individual marmosets varied how they changed these parameters; for example, some marmosets significantly decreased the duration of their calls whereas others significantly increased this parameter (Jorgensen and French 1998).

A sex difference in vocal stability was seen in collared doves, where females showed stability in their perch-coos over time and males changed several acoustic parameters (including coo stereotypy, modulation percentage, and number of overtones) between the ages of two and 18 months (Ballintijn and ten Cate 1997). Some parameters increased and others decreased, and the changes were very noticeable when the males were one year old (Ballintijn and ten Cate 1997). However, the fundamental frequencies and duration of male coos tended to remain stable over time (Ballintijn and ten Cate 1997).

Possible Factors Influencing Changes in Vocalizations

Changes in vocalizations over time could be caused by physical maturation or social influences (Elowson *et al.* 1992, Snowden and Hausberger 1997, Jorgensen and French 1998,

Boughman and Moss 2003). Physical maturation includes changes in the vocal apparatus, improvements of motor control, and changes in hormone levels—all of which can cause changes in acoustic parameters of vocalizations. For example, it is typical for larger individuals to produce vocalizations that are lower in frequency than those of smaller individuals (Boughman and Moss 2003). In addition, Hauser (1989) stated that changes in the vocal tract size appeared to change the acoustic structure of "wrr" calls in vervet monkeys (*Cercopithecus aethiops*), finding differences between these calls in infants and juveniles versus adults. Podos *et al.* (1995) found that song sparrows (*Melospiza melodia*) improved their motor control during song development with practice; the tonal quality of their vocalizations seemed to correspond to coordinated beak movement development. Similarly, Ballintijn and ten Cate (1997) suggested that a decrease in the number of harmonics produced by collared doves might be due to "motor practice." Changes in hormone levels have also been found to affect vocalizations. For example, the changes in perch-coos mentioned previously in only male collared doves were suggested to be due to testosterone levels (Ballintijn and ten Cate 1997). Social influences such as prolonged exposure to conspecific calls or the introduction of new conspecifics have been found to influence call structure in several species (Jorgensen and French 1998). Production learning, in which animals learn to produce sounds based on auditory input, has been documented in many species of birds, as well as in several species of marine mammals (Janik and Slater 2000) and elephants (Poole *et al.* 2005).

Manatees

Currently, little is known about if or how manatee calls may change over time. Sousa-Lima and da Silva (2001) found that the contours of Amazonian manatee vocalizations remained

stable over a period of at least four years, but nothing is known about stability, or lack thereof, over longer periods. In addition, although contour was stable, fundamental frequency range narrowed over time (there was an inverse correlation between fundamental frequency range and body size), and adult and subadult Amazonian manatees produced vocalizations of longer duration than those produced by calves (Sousa-Lima *et al.* 2002).

No work has been done on vocal stability in wild or captive Florida manatees. Manatee call stability is important to determine, because if manatee calls are found to have individually distinctive calls and yet they are not found to be stable, then this would suggest that calls are not used in individual recognition. On the other hand, if calls are found to be stable over either long or short time periods, there is greater potential that manatees use them in individual recognition. I tested the hypothesis that individual Florida manatee vocalizations are stable over short and long time periods in several ways. First, I compared recordings made between 1980-1987 (provided by O'Shea) to recordings I made of the same individuals between December 2002 and January 2004. Second, I compared calls of manatees recorded at least twice during their first few years of life and of adults that were recorded at least twice over short (1-3 year) time periods. Finally, I compared overall characteristics of calls of calves and adults in order to examine possible age differences. In each case, I investigated call parameters separately, to determine if certain parameters of calls changed while others remained stable.

Behaviors and Vocalizations

Some data exist regarding manatee vocalization rates and contexts of vocalizations. In the Blue Lagoon, Florida, Reynolds (1981) found that single animals and groups of two manatees (excluding mother-calf pairs) were relatively silent and produced very few

vocalizations. Mother-calf pairs were also relatively silent unless they were physically separated, frightened, fleeing an area, or joined by another manatee (Reynolds 1981). However, Alicea-Pou (2001) found that vocalizations were made constantly when mothers and calves were present. Both Reynolds (1981) and Alicea-Pou (2001) found that groups of more than two manatees made more vocalizations than smaller groups (one or two, excluding mother-calf pairs). Most vocalizations from groups of more than two manatees occurred after synchronous breathing or body-surfing (Reynolds 1981). Recently, Nowacek *et al*. (2003) found that Florida manatees at Crystal River produced a mean of 1.29 vocalizations/individual/minute, whereas three tagged Antillean manatees in Belize, either when alone or with others, were often silent for greater than 10 minutes and vocalized at rates of 0.09-0.75 vocalizations/individual/ minute. Phillips *et al.* (2004) found slightly lower vocalization rates of Florida manatees (1.09-1.88 calls/individual/5 minutes) at Homosassa Springs, but these rates were relatively consistent with the call rates (1-5 calls/individual/5 minutes depending on the behavior) found by Bengtson and Fitzgerald (1985).

Manatees have been reported to vocalize while playing, eating, engaging in sexual behavior, and when they become alarmed or frightened (Hartman 1979, Bengtson and Fitzgerald 1985, Ripple 1999, O'Shea *et al.* 2000, Alicea-Pou 2001, Sousa-Lima *et al.* 2002). Besides apparently conveying affective information in these situations, vocalizations also appear to function in maintaining contact between mothers and calves, and occur when an individual joins a group (Hartman 1979, Steel 1982, Bengtson and Fitzgerald 1985, Ripple 1999, O'Shea *et al.* 2000). Florida manatees at Blue Spring and the St. Johns River vocalized more often when cavorting (defined as socializing for the present research; see Appendix) and milling than when engaged in other behaviors such as bottom resting, feeding, or traveling (Bengtson and

Fitzgerald 1985); call rates increased when the intensity of social interactions increased. Alicea-Pou (2001) also found that more vocalizations occurred when manatees were socializing (with a peak during sexual behavior) than during resting, traveling, or feeding. Both Bengtson and Fitzgerald (1985) and Alicea-Pou (2001) found that manatees would stop vocalizing when approached by a canoe. Steel (1982) observed that vocalizations in Florida manatees were more likely to occur during activities such as playing, eating, and socializing than during other activities.

I attempted to examine how often and in which contexts Florida manatees vocalize. Specifically, I hypothesized that vocal rates vary depending on behavior. If this hypothesis is supported, this information could be used in conjunction with acoustic monitoring to determine which portions of a habitat may be preferred for various activities (such as feeding, mating, resting, etc.). In addition, acoustic monitoring could be used to provide insights into manatee behavior at night. Few studies have looked into nighttime behavior of manatees, although Bengtson (1981) reported that during winter months when the water temperatures were low, manatees seemed to have a daily cycle of leaving Blue Spring Run in the afternoon to feed at night. He did not find any other specific cycle during the rest of the year. Acoustic monitoring could increase our understanding of this understudied aspect of manatee behavior.

METHODS

Acoustic Recordings

Recordings of wild and captive Florida manatees were made in the 1980's by O'Shea with a stereo Uher 4400 Report Stereo IC reel-to-reel stereo tape recorder (Uher Informatik, Braunshweig, Germany; flat frequency response: 20-19000 Hz) and a US Navy model H-56

hydrophone (Underwater Sound Reference Division, Naval Undersea Warfare Center, New Port, Rhode Island; flat frequency response: 100-30000 Hz). Between November 2002 and May 2003 and between December 2003 and January 2004, under the auspices of the Sirenia Project's Federal Research Permit (MA791721-2), I made recordings of wild manatees at Florida's Blue Spring State Park (Figure 2) and of captive manatees at various public display facilities (Orlando's SeaWorld, Tampa's Lowry Park Zoo, Sarasota's Mote Marine Laboratory, Homosassa Springs Wildlife State Park, Ohio's Columbus Zoo, and Ohio's Cincinnati Zoo). Recordings in 2002-2004 were made with a High Tech, Inc. 94-SSQ hydrophone (High Tech, Inc., Gulfport, Mississippi; flat frequency response: 2-30000 Hz) and Marantz PMD-430 stereo cassette recorder (Marantz Company, Chatsworth, California; flat frequency response: 30-15000 Hz) or Panasonic AG-6400 stereo hi-fi VCR (Matsushita Electric Industrial Co., Ltd., Japan; flat frequency response: 20-20000 Hz). For recording wild manatees, the hydrophone was placed in the water either from land or off the side of a canoe. I made most recordings and observations of manatees in the wild from a canoe, and I tried focusing on small groups (e.g., 1-2 individuals), because it was easier for me to identify which manatee was vocalizing. For example, I was able to identify which manatee was vocalizing by following single animals for long periods of time and by following mother-calf pairs that separated from larger groups. Sometimes even the mothers and their calves separated for brief periods of time, during which vocalizations could be attributed to one or the other individual. Alicea-Pou (2001) found that he could record manatees that were over 10 meters away, and the manatees I recorded rarely, if ever, separated that far, but I was still able to make fairly certain identifications of vocalizations in some cases due to the relative amplitude of the sounds and locations of the manatees.

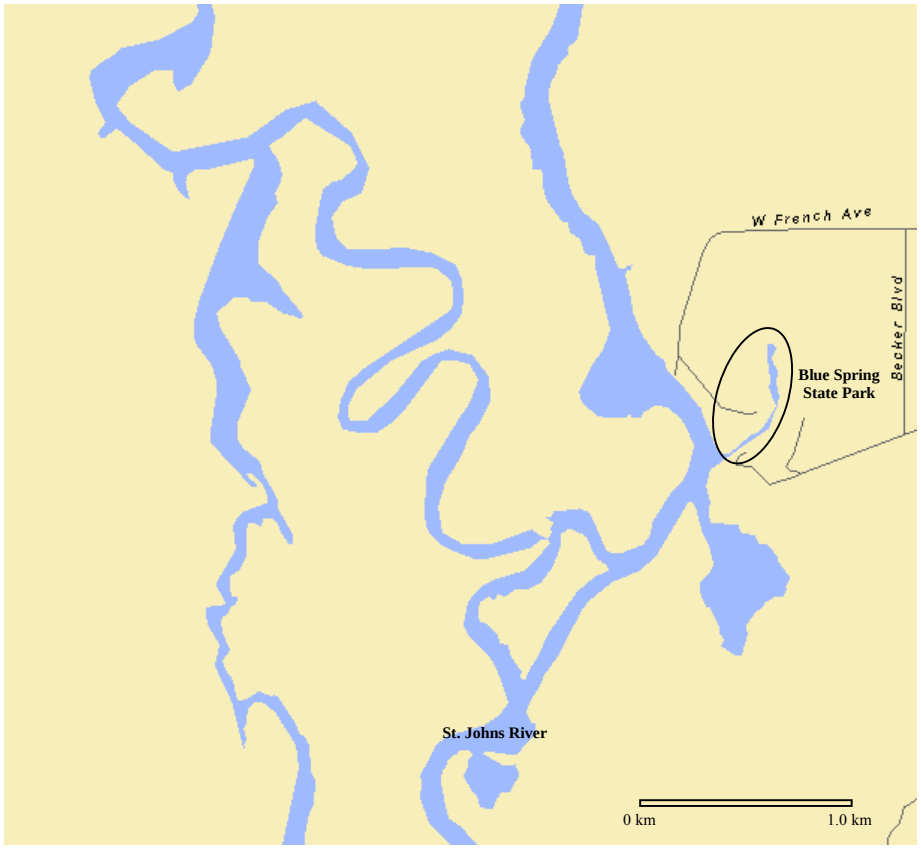

Figure 2. Map of the study area, Blue Spring State Park, Florida. The circle on the map on the right is indicating the location where the manatees were mainly observed and recorded.

For examining long-term stability of manatee vocalizations, I targeted the three manatees (Donna, Lucille, and Robin) that were recorded by O'Shea in the early 1980's that still visit Blue Spring State Park (Table 1). Identifications of these three animals were made in the field initially with the assistance of Ranger Wayne Hartley from Blue Spring State Park and then later by matching scar patterns on manatees to photographs and drawings of known individuals. Mother-calf pairs were also targeted, because prior research has shown that they tend to vocalize often, either for alarm or contact purposes (Hartman 1969, Reynolds and Odell 1991, Alicea-Pou 2001). These recordings contributed to the dataset on individually distinctive features in vocalizations and behavioral correlates of vocalizations.

Recordings of captive manatees were made by placing the hydrophone in the water and passively making observations from the side of the exhibit. No contact with the animals was made. In some instances, I was able to identify which captive manatee was vocalizing based on visual observation of wrinkling of their noses. Dugongs have been found to wrinkle their noses (an "alternate depression and elevation of the skin in this [frontal] region") when producing squeaks and chirp-squeaks (Anderson and Barclay 1995); temporarily captured manatees also have been observed wrinkling their noses when vocalizing (J. Powell, personal communication 2002). In two cases, animals were isolated from others, allowing for positive identification of the vocalizers. Two of the seven captive manatees O'Shea recorded in the 1980's are still alive and in public display facilities, and so these captive manatees (Gene and Rosie) were the primary target animals. As with the wild manatees, other manatees at various public display facilities were also recorded to increase the sample size of recorded individuals.

Table 1. Names and ID numbers of the 33 Florida manatees used in this study, their captive or wild status, age class, sex, number of days they were recorded, and number of vocalizations analyzed.

Manatee (ID #)	Captive/ Wild	Age Class	Sex	Days recorded	# of calls
Ann (BS189)	Wild	Adult	Female	1/27/03, 1/30/03	27
Brown (BS337)	Wild	Calf	Male	1/28/03, 2/13/03	36
C.C. (BS334)	Wild	Calf	Female	1/21/02, 1/25/03, 1/26/03	16
Calista (BS260)	Wild	Adult	Female	12/21/02, 1/12/03, 1/25/03, 1/26/03	19
Charlotte (SWFTm5828B)	Captive	Adult	Female	3/26/03	11
Cheryl (BS364)	Wild	Calf	Female	12/23/03, 12/26/03	31
Dawn (BS49)	Wild	Adult	Female	2/12/83, 2/16/83	13
Destiny (SWFTm0203B)	Captive	Adult	Female	2/18/03, 3/26/03	17
Donna (BS55)	Wild	Calf/ Adult	Female	2/12/83, 2/16/83, 12/19/02, 12/21/02, 12/22/02, 1/19/03, 1/28/03, 12/22/03	64
Fritz (BS359)	Wild	Calf	Male	1/2/04	14
Gene (992028)	Captive	Adult	Male	12/18/80, 11/8/02	33
Georgia (BS196)	Wild	Adult	Female	1/19/03, 1/28/03, 1/30/03	20
Goodland (RSW0207)	Captive	Subadult	Female	3/24/03, 3/25/03	12
Gray (BS338)	Wild	Calf	Male	1/28/03	24
Janice (BS332)	Wild	Calf/ Subadult	Female	1/26/03, 1/29/03, 12/23/03, 12/26/03, 12/29/03, 12/31/03	45
Jessica (BS131)	Wild	Adult	Female	1/26/03, 1/29/03	14
Judith (BS106)	Wild	Adult	Female	1/19/03, 1/25/03, 1/27/03, 1/28/03, 12/9/03, 12/27/03	31
June (BS92)	Wild	Adult	Female	12/22/02, 1/18/03, 12/9/03, 12/23/03, 12/30/03	23
Lorelei (SQM1)	Captive	Adult	Female	3/18/03, 3/19/03, 3/22/03	28
Loverboy (RSW0301)	Captive	Subadult	Male	3/24/03	12
Lucille (BS37)	Wild	Adult	Female	12/16/80, 2/4/81, 2/25/81, 3/13/81, 3/24/81, 3/26/81, 4/8/81, 2/24/82, 2/25/82, 4/21/83, 1/25/03, 1/29/03, 12/12/03, 12/28/03, 1/1/04	189
Luna (BS24)	Wild	Adult	Female	2/4/81, 3/13/81, 3/24/81, 4/8/81, 2/24/82, 2/25/82, 4/21/83	32
Macon (BS329)	Wild	Calf	Female	1/18/03, 1/28/03, 1/30/03, 12/31/03	48
Rita (SWFTm8233B)	Captive	Adult	Female	2/17/03, 3/26/03	29
Robin (BS35)	Wild	Calf/ Adult	Male	12/10/81, 2/23/81, 3/12/81, 4/3/81, 4/14/81, 12/12/03,12/26/03, 12/28/03	75
Rollin (BS340)	Wild	Adult	Male	2/5/03	18
Sal (RNW0209)	Captive	Calf	Male	3/24/03	32
San Remo (RNW0206)	Captive	Adult	Female	3/25/03	53
Scooter (SWFTm0201B)	Captive	Calf	Male	2/18/03, 3/26/03	33
Sherry (BS361)	Wild	Calf	Female	12/8/03, 12/30/03, 1/1/04	20
Steve (BS333)	Wild	Calf	Male	2/12/03	34
Stormy (BS304)	Wild	Adult	Male	2/26/03	43
Zeb (BS362)	Wild	Calf	Male	12/9/03, 12/22/03	20

Behavior Definitions

This study used the same classification scheme for behaviors that Koelsch (1997) used (see Appendix), with one exception. Instead of having a separate category for mating-like behavior [called cavorting by Koelsch (1997)], such behavior was combined into the social category with all interactions between two or more manatees (including nudging, bumping, and kissing). Other behavior categories included surface rest, bottom rest, mill, travel, feed, with boat, and play [as defined by Koelsch (1997)]. Brief descriptions of each of these categories are given here. Surface rest was defined as floating or basking at the surface with no change in location. Bottom rest was a low level of activity at the bottom of the water column (which can easily be seen at Blue Spring State Park as well as at most public display facilities due to the excellent water clarity) with no change in location and with surfacings at regularly spaced intervals for breaths. [When resting, manatees can stay submerged anywhere from one to 11 minutes, with adults usually staying submerged for longer durations than calves (Hartman 1979).] Milling was moving without directionality, and travel was directed movement. Feed [not described as a category in Koelsch (1997)] included visible food in a manatee's mouth and/or chewing, nursing, or grazing (defined here as mouth touching plants). With boat [not described as a category in Koelsch (1997)] was when a manatee approached my canoe and touched, rubbed, or stayed within two feet of my canoe. Finally, play was interacting with non-food objects such as pilings or lines (Koelsch 1997).

Behavioral Correlates

Data were collected using a group-follow (or focal-group sampling) protocol, as described by Mann (1999). Continuous behavioral observations were narrated onto a second

track of the tape, simultaneous with the acoustic recordings. During these observations, I noted how many manatees were in the group and the behavior of each individual in that group; I also noted when a manatee changed its behavior. Keeping track of behaviors of each individual manatee was possible because of their slow movements and the excellent visibility at Blue Spring State Park.

Data Analyses

Tapes were analyzed using a real time sound analysis program, Signal/RTSD (Version 3.0, Engineering Design, Belmont, Massachusetts). Nine parameters were measured for each vocalization: 1) fundamental frequency, 2) emphasized band, 3) duration, 4) emphasized band's frequency range, 5) interval from zero frequency to the fundamental frequency band, 6) interval from the fundamental frequency band to band two, 7) interval from band two to band three, 8) interval from band three to band four, and 9) number of complete bands less than or equal to 10 kHz (Figure 3). All nine measurements came from three different graphs: 1) a power spectrum (a plot of amplitude versus frequency), 2) a waveform (a plot of amplitude versus time), and 3) a spectrogram (a plot of frequency versus time). The fundamental frequency was the frequency in kHz of the first peak in the power spectrum (Figure 3a); the emphasized band, or the band with the most energy, was the frequency in kHz of the highest peak on the power spectrum (Figure 3a). I measured both the fundamental frequency and the emphasized band because these two parameters are not always the same in manatee calls, even though they are in Figure 3a. Duration was measured from the waveform and was defined to be the time duration over which nonzero call amplitude was present (Figure 3b). Spectrograms were used to measure the emphasized band's frequency range, which was the highest minus the lowest frequency for that

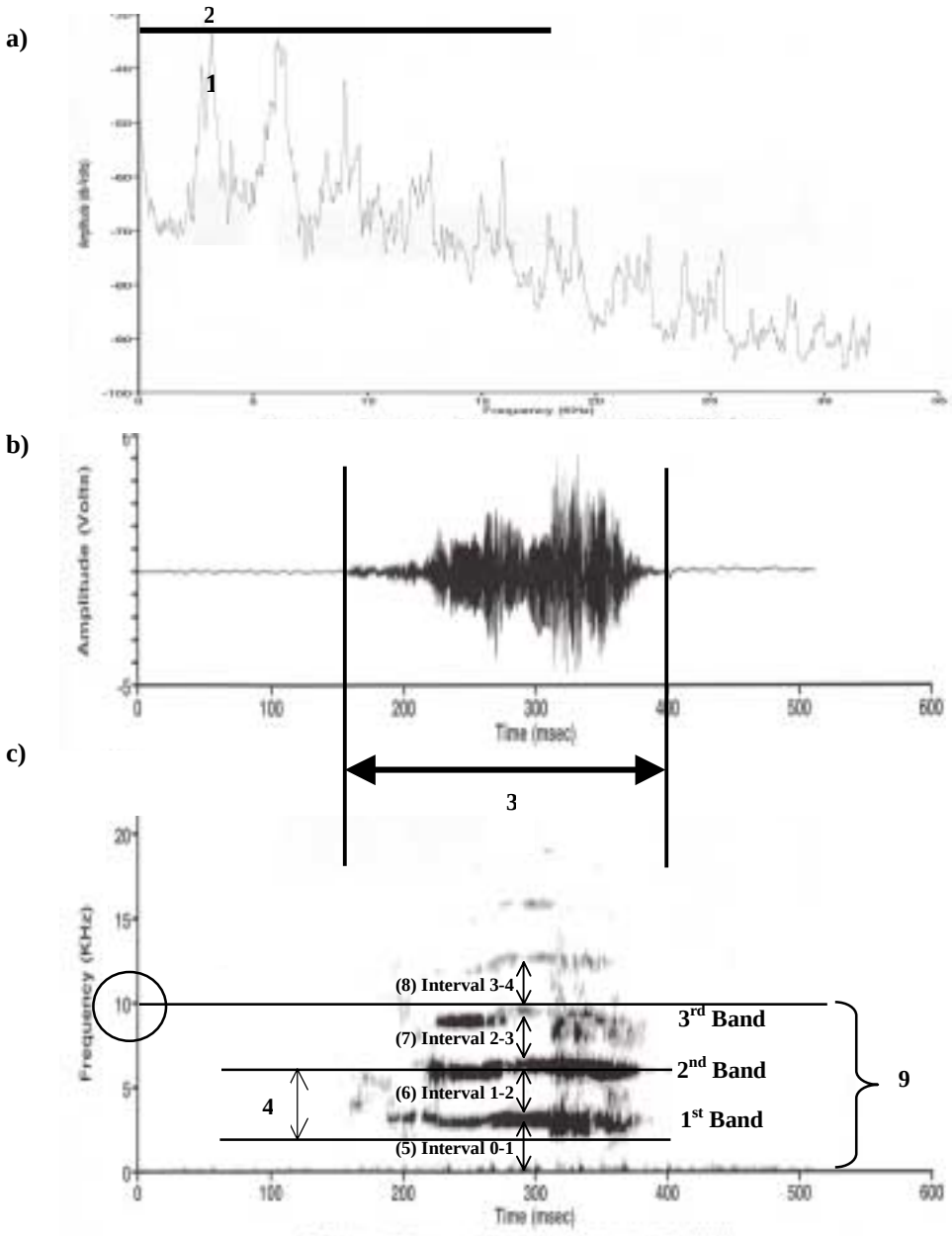

Figure 3. Methods for measuring the nine parameters from each vocalization. (a) Power spectrum showing how to measure (1) fundamental frequency and (2) emphasized band. (b) Waveform showing how to measure (3) duration. (c) Spectrogram showing how to measure (4) emphasized band's frequency range (Note: emphasized band in this example is the 1st band), (5-8) four interval measurements between bands, and (9) number of bands under 10 kHz. For the power spectrum, amplitude in dB-Volts is on the y-axis and frequency in kHz is on the x-axis. For the waveform, amplitude in Volts is on the y-axis and time in msec is on the x-axis. For the spectrogram, frequency in kHz is on the y-axis and time in msec is on the x-axis; settings are as in Figure 1.

band (Figure 3c); the intervals between the first four bands, which were taken from the middle of the vocalizations and started at zero frequency for the first interval measurement (Figure 3c); and the number of complete bands less than or equal to 10 kHz (Figure 3c). A high frequency cut-off of 10 kHz was used after examining calls from individuals, known to have multiple harmonics above 10 kHz, which I recorded at different distances from the hydrophone. For these individuals, I noted that 10 kHz was the highest frequency at which the number of harmonics recorded was not affected by any of the distances at which they were recorded. For the interval measurements, a band was considered as one of three possibilities: 1) the fundamental frequency, 2) a harmonic of the fundamental frequency, or 3) a subharmonic that was at least 50% of the duration of the fundamental frequency band[2]. Along the same lines, when determining the number of bands less than or equal to 10 kHz, subharmonics that were at least 50% of the duration of the fundamental frequency band were also included. Because all nine parameters described above could only be measured on vocalizations that had distinct bands, "noisy" calls without distinct bands were not used in the analyses for vocal signatures and vocal stability. Noisy calls were much less common in the recordings than the harmonic calls used in the analyses; less than approximately 5% of all vocalizations were noisy. The nine parameters were measured on a total of 1116 vocalizations after selecting vocalizations that were not noisy, not too faint due to distance, and not buried in background noise; no attempt was made to control for motivational state or activity at the time of vocalization.

Linear discriminant analyses were used to determine if there was a greater probability than expected by chance that vocalizations could be correctly classified to individual manatees when using the nine measured parameters. Linear discriminant analyses are tests that determine

[2] Subharmonics that were at least 50% of the call duration were used because they reached to the center of the call where the interval measurements were taken.

if groups (i.e., individual manatees, age classes, or sexes) differ from one another, and if so, which parameter(s) best discriminate observations (i.e., vocalizations) between groups (Huberty 1994). In these tests, classifiers are built for each group based on a "centroid" for each discriminant function. Centroids are mean values in k-dimensional space (with k as the number of parameters) and are determined by giving different parameters different weighted values depending on their importance in classifying between groups. The maximum number of discriminant functions is either the number of parameters used or one less than the number of groups being analyzed, whichever is less. Discriminant analyses classify observations to a group based on the centroid to which it is closest. For my study, classification rates were based on a "leave one out" cross-validated linear discriminant analysis (CVLDA), which leaves one observation (one vocalization in this case) out to build a classifier and then determines to which group that observation is categorized (Huberty 1994). Thus, the observation left out has no influence on its own classification. This cross-validation process of leaving one observation out at a time is done for every observation and determines the predictive accuracy and stability of the tests.

First, a CVLDA was performed on only the most recent (2002-2004) data, along with data from two adults—Dawn and Luna—whom O'Shea recorded in the 1980's but were not recorded after that time. This analysis provided both the number of calls assigned to the correct individual as well as the percentage of total calls assigned to the correct individual. Since there were 33 animals, the expected classification percentage was 1/33, or 3.03%. If the percentage was higher than expected by chance for a given individual, then the vocalizations of that individual were considered to have individually distinctive features. To test the hypothesis that Florida manatee vocalizations are stable over long time periods, a CVLDA was performed with

all data (using vocalizations from the 1980's and 2002-2004). Vocalizations were considered stable if a higher overall percentage of calls was assigned to individuals recorded both in the 1980's and 2002-2004 when data from the 1980's were added.

One-way, repeated measures ANOVAs were performed on each of the nine parameters for individual manatees to determine if certain parameters change over either long (19+ year) or short (1-3 year) time periods. The repeated measures were an individual's vocalizations recorded on the same day. In cases where data were not normal (based on visual inspection of histograms), log-log and log-square root transformations were used to normalize the data. Gene was recorded on only two different days, and thus two-sided Wilcoxon signed-rank tests (instead of ANOVAs) were performed on each of his nine parameters to determine if any parameters changed over 19+ years. Plots of recording date versus acoustic parameter were also made for each individual for which ANOVAs and Wilcoxon signed-rank tests were performed to aid in visualizing the data.

Three different "categories" of manatees were analyzed. The first category included the four animals recorded both in the 1980's and between 2002-2004 (Donna, Gene, Lucille, and Robin), and was analyzed to determine if parameters changed over a long (19+ year) time period. Of these four, Gene was the only manatee who was an adult in the 1980's when O'Shea first recorded him; the other three manatees (Donna, Lucille, and Robin) were all calves [defined as in Hartman (1979), which included those manatees seen associating with their mothers[3]]. The second category included calves that were recorded when they were less than one year old and at least once more when they were between one and two years of age, even if they were not considered calves after that first recording period (Janice, Lucille, Macon, and Robin). In one

[3] Also included in the CVLDAs as calves were orphans or dependent calves less than one year old, which included Sal, Scooter, and Zeb.

case (Lucille), the manatee was recorded again between the ages of one and two as well as between the ages of two and three, each time associating with her mother (Luna). Janice was seen associating with her mother during the first field season and thus considered a calf that year; however, during the second field season, she never associated with her mother and thus was considered a subadult [defined in this study as a manatee not seen associating with its mother but not yet sexually mature; sexual maturity occurs approximately between 6-10 years of age in manatees (Reynolds and Odell 1991)]. The third category included adults (sexually mature animals[4]) that were recorded in at least two separate field seasons over a short (1-3 year) time period (Judith, June, Lucille, and Luna).

Additionally, CVLDAs were performed to determine if there was an overall difference in vocalizations between calves and adults or between male and female calves. Subadults were not compared because of the small sample size of manatees that fit this category. Male and female adults were not compared, because very few adult males were recorded. One-way ANOVAs were also performed on all nine parameters individually to determine if there were significant differences between calves and adults or between male and female calves. Log-log and log-square root transformations were used to normalize non-normal data when possible.

Call rates, defined as the number of all manatee vocalizations (noisy and harmonic) per minute, were correlated with behavioral data to test the hypothesis that Florida manatee vocalization rates vary depending on behavior. Rates of vocalizations in various activities were determined using the main behavior for each manatee of a focal follow group from every minute. Vocal rates were modeled as a linear function of group size and behavioral category (and potential interaction between these) using a generalized linear model with negative binomial

[4] Also included in the CVLDAs as an adult was San Remo, who was not yet sexually mature but had reached puberty (personal communication from Lowry Park Zoo veterinarian, Dr. David Murphy 2005).

parent distribution and log link function (generalized linear models are discussed in Agresti 2002 and Hardin and Hilbe 2003). This model was used to determine the call rates per minute per manatee for all behaviors, the call rates per minute as a function of group size for all behaviors, and which behaviors had significantly different rates than other behaviors. This statistical test took into account repeated measures (observations from one focal group), and it also took into account the fact that different manatees were engaged in different behaviors by weighting behaviors according to the number of manatees doing them. This weighted number for each behavior was then used to determine vocalization rates during each behavior. Because of the conservative nature of this test (the actual type I error rates were much less than nominally stated, meaning the test was more stringent than the nominal type I error rate would suggest), alpha was set at 0.10. The total number of "manatee minutes" was also calculated for each behavioral category by totaling the number of minutes that each individual manatee spent in a particular behavior.

RESULTS

Three of the nine wild manatees recorded in the 1980's (Donna, Lucille, and Robin) still visit Blue Spring State Park. I spent a total of 44 field days at Blue Spring State Park between December 16, 2002 and February 26, 2003, and collected approximately 129 hours of recordings. During this time, all three manatees were found, followed, and recorded, and vocalizations were positively identified for two of the three animals. I identified Lucille's vocalizations by following her as she traveled alone down the St. Johns River, and I identified Donna and her twins' (Brown and Gray) likely vocalizations on several different occasions during which they were isolated from other manatees. Donna always associated with one twin,

whereas the other twin often milled around without its mother; recordings were obtained in these contexts as well as when all three were together. I was not able to obtain sufficient recordings of the third manatee, Robin, so I made another attempt between December 7, 2003 and January 3, 2004, during which time I recorded a few of his vocalizations when he traveled and milled alone in the St. Johns River. During this second field season, I spent 20 field days at Blue Spring State Park and made approximately 64 hours of recordings, which included recordings of some of the same individuals that I had recorded during the first field season, as well as several new individuals.

Only two (Gene and Rosie) of the seven captive manatees recorded in the 1980's are still alive and in captivity. Both of these manatees were recorded, although Rosie never vocalized during three days of recording. I recorded Gene in isolation at the Columbus Zoo, which assured correct identification of his vocalizations (although he was not very vocal). I spent a total of 13 days at the six aforementioned public display facilities and collected approximately 85 hours of recordings.

Overall, while working at Blue Spring State Park and at the public display facilities, recordings of 27 new manatees (9 captive, 18 wild) were obtained for examining the hypotheses that vocalizations are individually distinctive and that vocal rates are context dependent. Additionally, vocalizations of two manatees that were recorded by O'Shea in the 1980's but not rerecorded (Dawn and Luna) were included in the analysis; thus, the vocalizations of a total of 33 manatees were analyzed (Table 1).

The CVLDA using only the 2002-2004 data plus the vocalizations from Dawn and Luna from the 1980's (a total of 863 vocalizations[5]) assigned a higher percentage of calls than expected by chance to 30 out of 33 individuals (all but Goodland, Janice, and Loverboy), although C.C., Cheryl, Georgia, June, and Steve all had rather low percentages (<20%; Table 2). Figure 4 shows spectrograms of one call from each individual, illustrating the individual variability among calls. However, many calls were misclassified as another animal; those misclassified at a level of 20%[6] or greater were: Steve as Brown (20.6%), Zeb as Brown (20%), Goodland as Calista (25%), Georgia as Fritz (30%), Stormy as Gene (22.5%), Sherry as Gray (20%), Ann as Judith (22.2%), C.C. as Macon (25%), June as Rita (21.7%), Judith as Robin (25.8%), Janice as Sal (20%), and Cheryl as Zeb (29.0%; Table 2).

The CVLDA using all of the data (from the 1980's and 2002-2004—a total of 1058 vocalizations; see footnote #5), which was done to examine long-term stability in vocalizations of Donna, Gene, Lucille, and Robin, resulted in a higher percentage of calls correctly assigned to only one of the four (Donna; Table 3). The percentage of calls correctly assigned to Donna increased from 42.1% when only the 2002-2004 vocalizations were used to 48.4% when all vocalizations were used. Lower percentages of calls were correctly assigned to Gene, Lucille, and Robin (Table 3); Gene decreased from 50% to 18.2%, Lucille from 68.8% to 25.9%, and Robin from 53.3% to 42.7%. Although lower percentages were classified for three of the animals, higher percentages than expected by chance still were assigned to all four individuals.

[5] The discriminant analyses required measured values for all parameters; thus, only vocalizations with at least four bands could be used. This eliminated 37 vocalizations when using only the most recent data (plus the calls of Dawn and Luna), and eliminated 58 vocalizations when using all of the data.

[6] I chose 20% as a value that seemed high enough to potentially be biologically significant to the animals (see discussion).

Table 2. Results from the cross-validated linear discriminant analysis using only the 2002-2004 data plus vocalizations from Dawn and Luna from the 1980's. These numbers represent percentages of calls either correctly or incorrectly classified to an individual. The percentages of calls correctly classified to individuals are in boldface along the diagonal. To determine where an individual's calls were classified, find the row with the name of the desired individual and read across. If there is a percentage greater than 0%, at least one call was classified to the animal whose name is at the top of that specific column. Table 1 gives the total number of calls (from the 1980's and 2000's) for each individual used in this study; however, if a value could not be recorded for at least one call parameter, this call was not included in the discriminant function analysis. A higher percentage of calls than would have been expected by chance (3.03%) was assigned to the correct individual for 30 out of 33 manatees.

Maradee	Ann	Brown	C.C.	Calista	Charlotte	Cheryl	Dawn	Destiny	Donna	Fritz	Gene	Georgia	Goodbird	Gray	Janice	Jessica	Judith	June	Lorelei	Loverboy	Lucille	Luna	Macon	Rita	Robin	Robin	Sal	San Remo	Scooter	Sherry	Steve	Stormy	Zeb
Ann	**66.7**	0	0	11.1	0	0	0	0	0	0	0	0	0	0	0	0	22.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown	0	**69.4**	2.8	0	0	5.6	0	0	0	0	0	0	0	0	0	0	0	0	2.8	0	0	0	2.8	0	0	0	0	0	0	0	2.8	0	13.9
C.C.	0	0	**6.3**	0	6.3	0	0	0	0	0	0	6.3	0	6.3	0	12.5	0	0	0	0	0	0	25	0	6.3	0	0	0	0	6.3	12.5	0	12.5
Calista	16.7	0	0	**38.9**	0	0	5.6	5.6	0	0	0	0	5.6	0	11.1	0	0	0	0	5.6	0	0	0	0	0	0	0	0	0	0	0	11.1	0
Charlotte	0	0	9.1	0	**54.6**	0	0	0	0	0	0	18.2	0	0	0	9.1	0	9.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cheryl	0	9.7	6.5	0	0	**12.9**	0	0	6.5	0	3.2	3.2	0	3.2	0	0	0	0	3.2	3.2	0	0	0	0	0	0	0	3.2	0	0	16.1	0	29.0
Dawn	0	0	0	0	0	0	**30.8**	7.7	0	0	0	7.7	0	0	0	7.7	0	15.4	0	0	0	7.7	7.7	0	0	0	0	7.7	0	7.7	0	0	0
Destiny	0	0	0	0	0	0	0	**94.1**	5.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Donna	0	0	0	0	0	2.6	7.9	0	**42.1**	0	0	0	0	0	0	0	0	10.5	0	2.6	0	5.3	0	13.2	0	0	0	13.2	0	2.6	0	0	0
Fritz	0	0	0	0	0	7.1	0	14.3	0	**71.4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.1	0	0	0	0	0
Gene	0	7.1	0	0	0	0	0	0	0	0	**50**	0	0	0	7.1	0	0	0	14.3	0	14.3	0	7.1	0	0	0	0	0	0	0	0	0	0
Georgia	0	0	10	0	5	0	0	5	0	30	0	**5**	0	15	0	15	0	0	0	0	0	0	5	0	0	0	0	0	0	10	0	0	0
Goodbird	0	0	0	25	0	0	0	16.7	0	0	8.3	0	**0**	0	0	0	16.7	0	0	8.3	0	0	0	0	8.3	0	8.3	0	0	0	0	8.3	0
Gray	0	0	0	0	0	8.3	0	0	0	0	0	0	0	**33.3**	0	0	0	0	16.7	0	0	4.2	8.3	0	12.5	0	0	0	0	12.5	4.2	0	0
Janice	8.9	0	6.7	0	0	0	0	0	0	0	6.7	0	0	2.2	**2.2**	13.3	2.2	0	0	0	0	0	8.9	0	6.7	8.9	20	0	4.4	4.4	0	4.4	0
Jessica	0	0	0	0	7.1	0	0	0	0	0	0	14.3	0	0	0	**78.6**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Judith	12.9	0	0	3.2	0	0	0	0	0	0	0	0	3.2	0	0	0	**54.8**	0	0	0	0	0	0	0	25.8	0	0	0	0	0	0	0	0
June	0	0	0	0	13.0	0	0	0	8.7	0	8.7	0	0	0	0	4.4	0	**13.0**	4.4	0	0	0	4.4	21.7	0	0	0	17.4	0	0	4.4	0	0
Lorelei	0	3.6	3.6	0	0	0	0	3.6	0	0	0	0	0	0	0	0	0	10.7	**50**	0	0	0	3.6	0	0	0	0	0	0	10.7	10.7	3.6	0
Loverboy	0	8.3	0	8.3	0	0	8.3	8.3	8.3	8.3	0	0	0	8.3	0	0	0	0	0	**0**	0	16.7	0	0	8.3	0	0	0	0	0	0	16.7	0
Lucille	1.3	0	0	1.3	0	0	0	0	0	0	5	0	0	0	8.8	0	1.3	0	0	0	**66.8**	1.3	0	0	3.8	2.5	0	0	0	0	0	6.3	0
Luna	0	0	0	0	0	0	6.3	0	0	0	0	0	0	12.5	0	0	0	6.3	0	6.3	0	**62.5**	0	6.3	0	0	0	0	0	0	0	0	0
Macon	0	0	2.1	0	2.1	8.5	0	4.3	0	0	4.3	0	2.1	2.1	0	0	0	0	4.3	2.1	0	0	**59.6**	0	0	0	2.1	0	0	4.3	0	0	2.1
Rita	0	0	0	0	0	0	0	0	3.5	0	0	0	0	0	0	0	0	3.5	0	0	0	0	0	**89.7**	0	0	0	3.5	0	0	0	0	0
Robin	13.3	0	0	0	0	0	0	0	0	0	0	0	0	13.3	6.7	0	6.7	0	0	0	0	6.7	0	0	**53.3**	0	0	0	0	0	0	0	0
Robin	5.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.1	0	0	0	0	0	0	0	0	**72.2**	11.1	0	0	0	0	0	0
Sal	3.1	0	0	0	0	0	3.1	0	0	0	3.1	0	0	6.3	12.5	0	6.3	0	0	0	0	0	0	0	3.1	6.3	**53.1**	0	0	3.1	0	0	0
San Remo	0	0	1.9	0	0	0	1.9	0	15.1	0	0	0	0	0	0	0	0	17.0	0	0	0	0	0	9.4	0	0	0	**54.7**	0	0	0	0	0
Scooter	6.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.0	0	0	0	0	0	0	0	0	6.1	0	0	0	**84.9**	0	0	0	0
Sherry	0	5	0	0	0	5	5	0	0	0	15	5	0	20	0	5	0	0	10	0	0	5	0	0	0	0	0	0	0	0	**25**	0	0	0
Steve	0	20.6	8.8	0	0	14.7	0	0	0	0	0	0	0	11.8	0	0	0	0	11.8	0	0	0	2.9	0	0	0	0	0	0	5.9	**17.7**	0	5.9
Stormy	0	0	0	7.5	0	0	0	0	0	0	22.5	0	0	0	0	2.5	5	0	0	0	7.5	0	0	0	0	2.5	0	0	0	0	0	**52.5**	0
Zeb	0	20	0	0	0	15	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	5	0	0	0	0	0	0	0	10	0	**40**

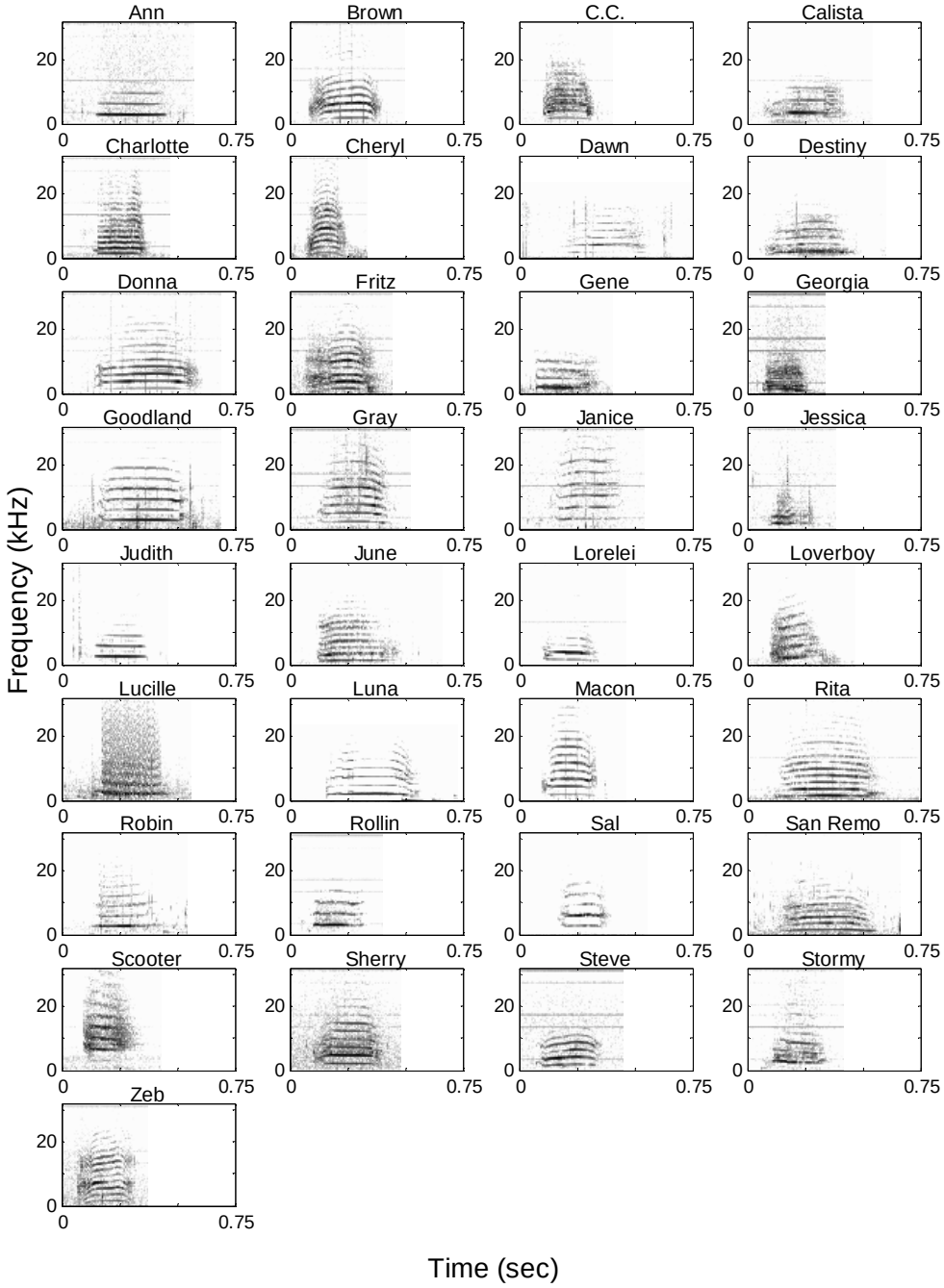

Figure 4. Spectrograms of a vocalization of each of the 33 manatees recorded, showing individual variability in contours and other parameters.

Table 3. Results from the cross-validated linear discriminant analysis using all of the data (from the 1980's and 2002-2004). These numbers represent percentages of calls either correctly or incorrectly classified to an individual. The percentages of calls correctly classified to individuals are in boldface along the diagonal. To determine where an individual's calls were classified, find the row with the name of the desired individual and read across. If there is a percentage greater than 0%, at least one call was classified to the animal whose name is at the top of that specific column. Table 1 gives the total number of calls (from the 1980's and 2000's) for each individual used in this study; however, if a value could not be recorded for at least one call parameter, this call was not included in the discriminant function analysis. Four animals were recorded in the 1980's and in 2002-2004; if their calls were stable over this time period, then higher percentages of calls should be assigned to them than were assigned when using only the 2002-2004 data (Table 2). However, an overall higher percentage of calls was correctly assigned only to Donna, whereas lower percentages were assigned to Gene, Lucille, and Robin.

Maradex	Ann	Brown	C.C.	Calista	Charlotte	Cheryl	Dawn	Destiny	Donna	Fritz	Gene	Georgia	Goodbird	Gray	Janice	Jessica	Judith	June	Lorelei	Loverboy	Lucille	Luna	Macan	Rita	Robin	Robin	Sol	San Remo	Scooter	Sherry	Steve	Stormy	Zeb
Ann	**66.7**	0	0	11.1	0	0	0	0	0	0	0	0	0	0	0	0	22.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown	0	**69.4**	2.8	0	0	5.6	0	0	0	0	0	0	0	0	0	0	0	0	2.8	0	0	0	2.8	0	0	0	0	0	0	0	0	0	16.7
C.C.	0	0	**12.5**	0	6.3	0	0	0	0	0	0	6.3	0	6.3	0	12.5	0	0	0	0	0	0	18.8	0	6.3	0	0	0	0	12.5	12.5	0	6.3
Calista	16.7	0	0	**44.4**	0	0	5.6	0	0	0	0	0	5.6	0	5.6	0	0	0	0	0	5.6	0	0	0	5.6	0	0	0	0	0	0	11.1	0
Charlotte	0	0	18.2	0	**54.6**	0	0	0	0	0	0	18.2	0	0	0	9.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cheryl	0	9.7	0	0	6.5	**12.9**	0	0	3.2	0	0	3.2	0	3.2	0	0	0	0	3.2	9.7	0	0	3.2	0	0	0	0	0	0	0	12.9	0	32.3
Dawn	0	0	0	0	0	0	**38.5**	7.7	0	7.7	0	7.7	0	0	0	0	0	15.4	0	0	0	7.7	7.7	0	0	0	0	7.7	0	0	0	0	0
Destiny	0	0	0	0	0	0	0	**94.1**	5.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Donna	0	4.7	0	0	0	3.1	4.7	1.6	**48.4**	1.6	0	1.6	0	0	0	0	0	3.1	0	1.6	0	4.7	0	7.8	0	0	0	12.5	0	0	3.1	0	1.6
Fritz	0	0	0	0	0	7.1	0	14.3	0	**71.4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.1	0	0	0	0	0
Gene	6.1	3.0	0	3.0	3.0	0	0	3.0	0	0	**18.2**	0	0	3.0	0	0	3.0	0	9.1	0	3.0	0	15.2	0	18.2	0	9.1	0	0	0	0	3.0	0
Georgia	0	0	10	0	0	0	0	5	0	25	0	**10**	0	0	0	20	0	0	0	0	0	0	5	0	15	0	0	0	0	10	0	0	0
Goodbird	0	0	0	33.3	0	0	0	8.3	0	0	8.3	0	**0**	0	0	0	25	0	0	8.3	0	0	0	0	0	0	8.3	0	0	0	0	8.3	0
Gray	0	0	0	0	0	4.2	0	0	0	0	4.2	0	0	**41.7**	0	0	0	0	20.8	0	0	4.2	4.2	0	4.2	0	0	0	0	12.5	4.2	0	0
Janice	13.3	0	8.9	0	0	0	0	0	0	0	6.7	0	0	2.2	**0**	13.3	2.2	0	0	0	0	0	6.7	0	2.2	8.9	17.8	0	4.4	8.7	0	8.7	0
Jessica	0	0	0	0	7.1	0	0	0	0	0	0	14.3	0	0	0	**78.6**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Judith	19.4	0	0	3.2	0	0	0	0	0	0	3.2	0	0	0	0	0	**71.0**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
June	0	0	0	0	13.0	0	0	0	4.4	0	4.4	0	0	0	0	4.4	0	**13.0**	4.4	0	0	0	4.4	26.1	0	0	0	17.4	0	0	8.7	0	0
Lorelei	0	3.6	0	0	0	0	0	3.6	0	0	0	0	0	0	0	0	0	10.7	**50**	3.6	0	0	3.6	0	0	0	0	0	0	10.7	10.7	3.6	0
Loverboy	0	8.3	0	8.3	0	0	8.3	8.3	0	8.3	0	0	0	16.7	0	0	0	0	0	**0**	0	8.3	0	8.3	8.3	0	0	0	0	0	0	16.7	0
Lucille	5.9	4.7	0	3.5	0	0.6	0	0.6	0	0	6.5	0	0	1.8	3.5	0	3.5	0	5.3	1.8	**25.9**	1.2	1.2	0	3.5	7.1	8.2	0	0.6	0	0.6	14.1	0
Luna	0	0	0	0	0	0	6.3	0	0	0	0	0	0	12.5	0	0	0	6.3	0	6.3	0	**62.5**	0	6.3	0	0	0	0	0	0	0	0	0
Macan	0	0	2.1	0	2.1	8.5	0	4.3	0	0	2.1	0	2.1	2.1	0	0	0	0	2.1	2.1	0	0	**63.8**	0	2.1	0	0	0	0	4.3	0	0	2.1
Rita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.9	0	0	0	0	0	**89.7**	0	0	0	3.5	0	0	0	0	0
Robin	6.7	2.7	0	0	0	2.7	0	2.7	0	0	5.3	0	0	5.3	14.7	0	5.3	0	0	5.3	1.3	1.3	0	0	**42.7**	0	2.7	0	0	1.3	0	0	0
Robin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.6	0	11.1	0	0	0	5.6	0	0	0	0	**72.2**	5.6	0	0	0	0	0	0
Sol	3.1	0	0	0	0	0	3.1	0	0	0	3.1	0	0	6.3	12.5	0	0	0	0	0	12.5	0	0	0	12.5	3.1	**40.6**	0	0	3.1	0	0	0
San Remo	0	0	1.9	0	0	0	5.7	0	9.4	0	0	0	0	1.9	0	0	0	17.0	0	0	0	0	0	15.1	0	0	0	**49.1**	0	0	0	0	0
Scooter	6.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.1	0	0	0	3.0	0	0	0	3.0	0	0	0	**81.8**	0	0	0	0
Sherry	0	5	0	0	0	5	5	0	0	0	0	5	0	25	0	5	0	0	15	0	0	5	0	0	0	0	0	0	0	**30**	0	0	0
Steve	0	17.7	8.8	0	2.9	17.7	0	0	0	0	0	0	0	11.8	0	0	0	0	11.8	0	0	0	2.9	0	0	0	0	0	0	5.9	**14.7**	0	5.9
Stormy	0	0	0	2.5	0	0	0	0	0	0	2.5	0	2.5	0	5	2.5	5	0	0	0	5	0	0	0	0	2.5	0	0	0	0	0	**72.5**	0
Zeb	0	20	0	0	5	15	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	5	0	0	0	0	0	0	0	10	0	**35**

There were nine discriminant functions (equal to the number of parameters) for the CVLDAs that tested for individual distinctiveness and stability over time, with the first function being the most important and the ninth being the least important in terms of discriminating observations (calls) between groups (i.e., individuals). For each analysis, four of these nine discriminant functions were significant. Discriminant functions were considered significant if their Eigenvalues were above or at a plateau when graphically displaying all nine Eigenvalues versus their discriminant function number. The most important parameter(s), if any, for each function (those that best defined a group and were above a 0.7 value, representing the correlation between a specific parameter and the discriminant function) are listed in Table 4.

One-way ANOVAs for each of the nine parameters yielded a variety of significant findings. One-way ANOVAs could not be completed for parameters that did not have enough distinct observations or that were not normally distributed and could not be normalized with log-log or log-square root transformations. These cases are noted with a "*" in Tables 5-8; all other data will be outlined here. The first category of animals included those that had been recorded over 19+ years (Donna, Lucille, Robin, and Gene; Tables 5-6, Figures 5-8). The frequency of the emphasized band of Donna's vocalizations significantly decreased and duration significantly increased between February 12, 1983 and December 22, 2003 (Table 5 and Figure 5). The frequency of the emphasized band of Lucille's vocalizations also significantly decreased between December 16, 1980 and January 1, 2004, as did her frequency range, interval 0-1, interval 2-3, and interval 3-4 (Table 5 and Figure 6). The frequency of the emphasized band of Robin's vocalizations also significantly decreased between February 23, 1981 and December 28, 2003, although his fundamental frequency significantly increased (Table 5 and Figure 7). ANOVAs could not be performed on the vocalizations of Gene (the only manatee that was an

Table 4. Most important parameters for each significant discriminant function (those parameters that played the largest role in defining groups) for the cross-validated linear discriminant analyses on: the 2002-2004 data plus Luna and Dawn, all of the data (2002-2004 plus 1980's), adults versus calves, and female versus male calves. Discriminant functions are in decreasing order of importance when discriminating between groups (i.e., individuals, age classes, or sexes). "*" indicates those functions not significant.

Discriminant Function	2002-2004 data + Luna and Dawn	All of the data	Adults vs. Calves	Female vs. Male Calves
1	Fundamental frequency, interval 0-1	Fundamental frequency, interval 0-1	Emphasized band	Emphasized band's frequency range
2	Emphasized band's frequency range	Emphasized band's frequency range	*	*
3	(none)	(none)	*	*
4	(none)	(none)	*	*

Table 5. *p* values from one-way, repeated measures ANOVAs on vocal parameters of individual manatees recorded over at least 19 years. Cases for which there were not enough distinct observations or in which data could not be normalized are marked with a "*". In some cases, not all parameters could be measured for each vocalization (i.e., if there were less than four bands), which resulted in different n values for different parameters.

Manatee	Emphasized Band	Fundamental Frequency	Duration	Frequency Range	# of Bands <10 kHz	Interval 0-1	Interval 1-2	Interval 2-3	Interval 3-4
Donna									
p	0.0378	0.3423	0.0550	*	*	0.2173	*	*	*
F	7.04	1.06	5.65			1.90			
num df	1	1	1			1			
den df	6	6	6			6			
n	64	64	64			64			
Lucille									
p	<0.0001	0.3260	0.9817	0.0407	*	0.0112	0.3066	0.0151	0.0300
F	37.07	1.04	0.00	5.16		8.71	1.13	7.83	5.93
num df	1	1	1	1		1	1	1	1
den df	13	13	13	13		13	13	13	13
n	189	189	189	189		170	170	170	170
Robin									
p	0.0027	0.0514	0.2964	0.3260	*	*	*	*	*
F	24.02	5.89	1.31	1.14					
num df	1	1	1	1					
den df	6	6	6	6					
n	75	75	75	75					

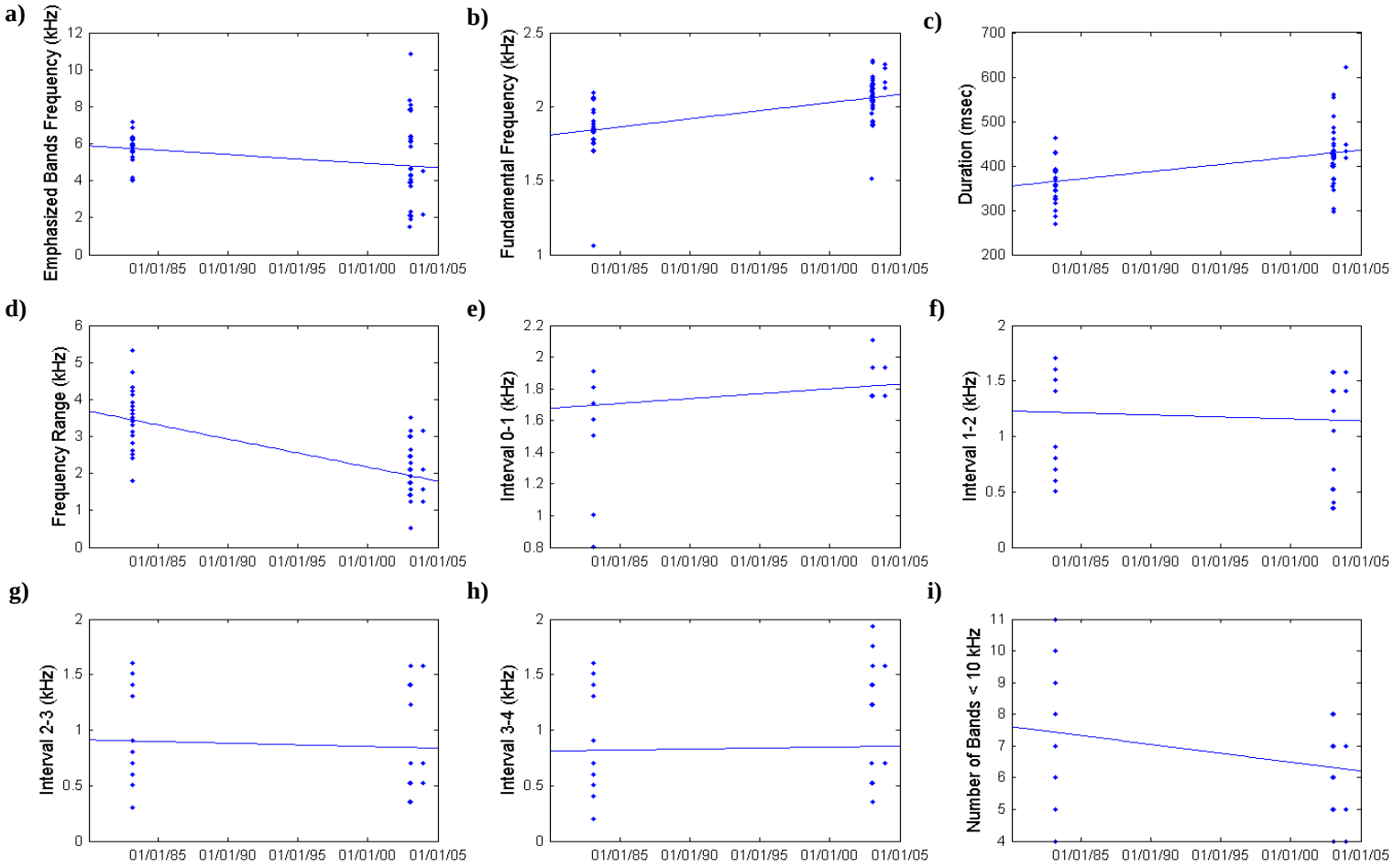

Figure 5. Plots of recording date versus acoustic parameter for Donna's vocalizations for every day she was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

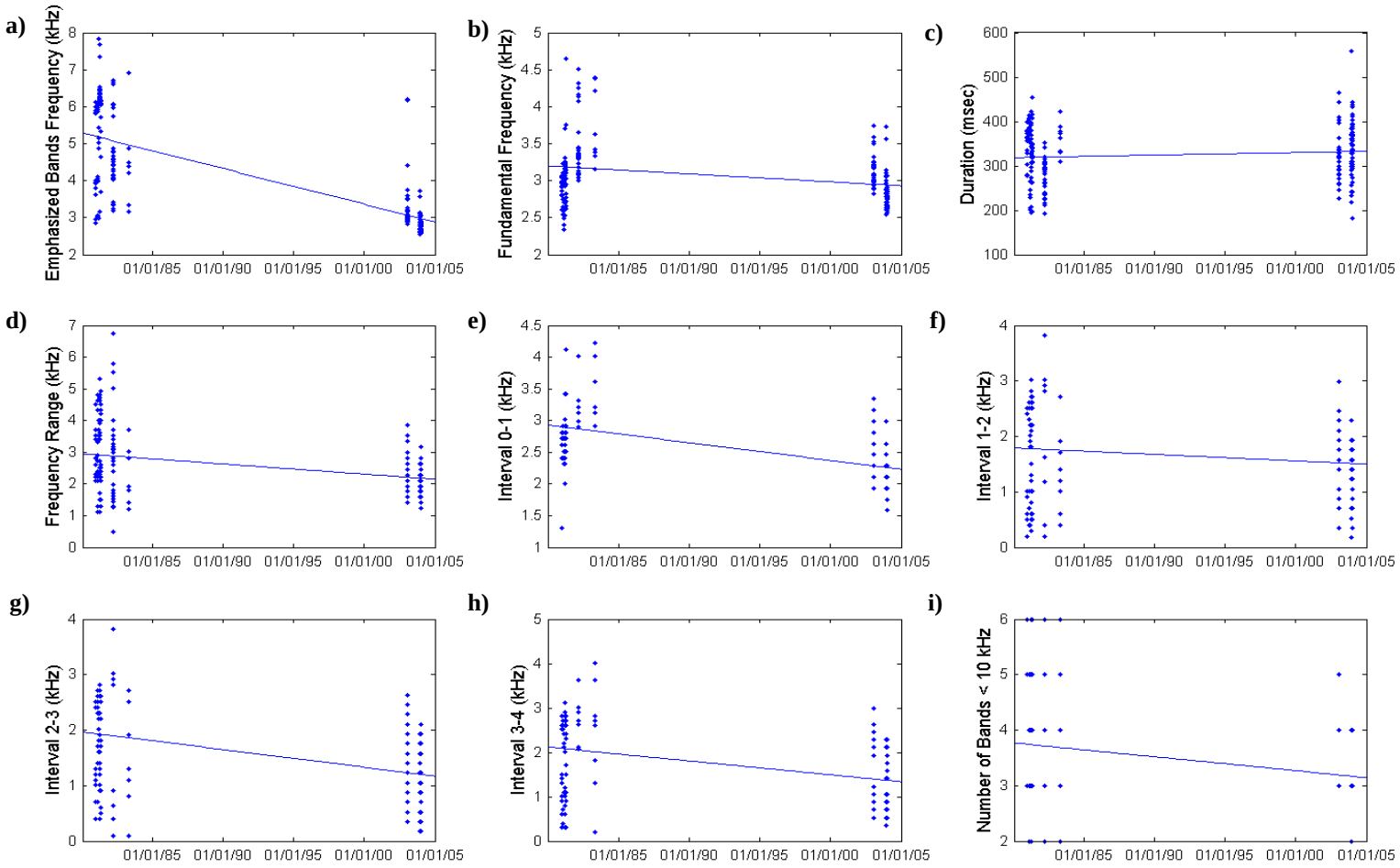

Figure 6. Plots of recording date versus acoustic parameter for Lucille's vocalizations for every day she was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

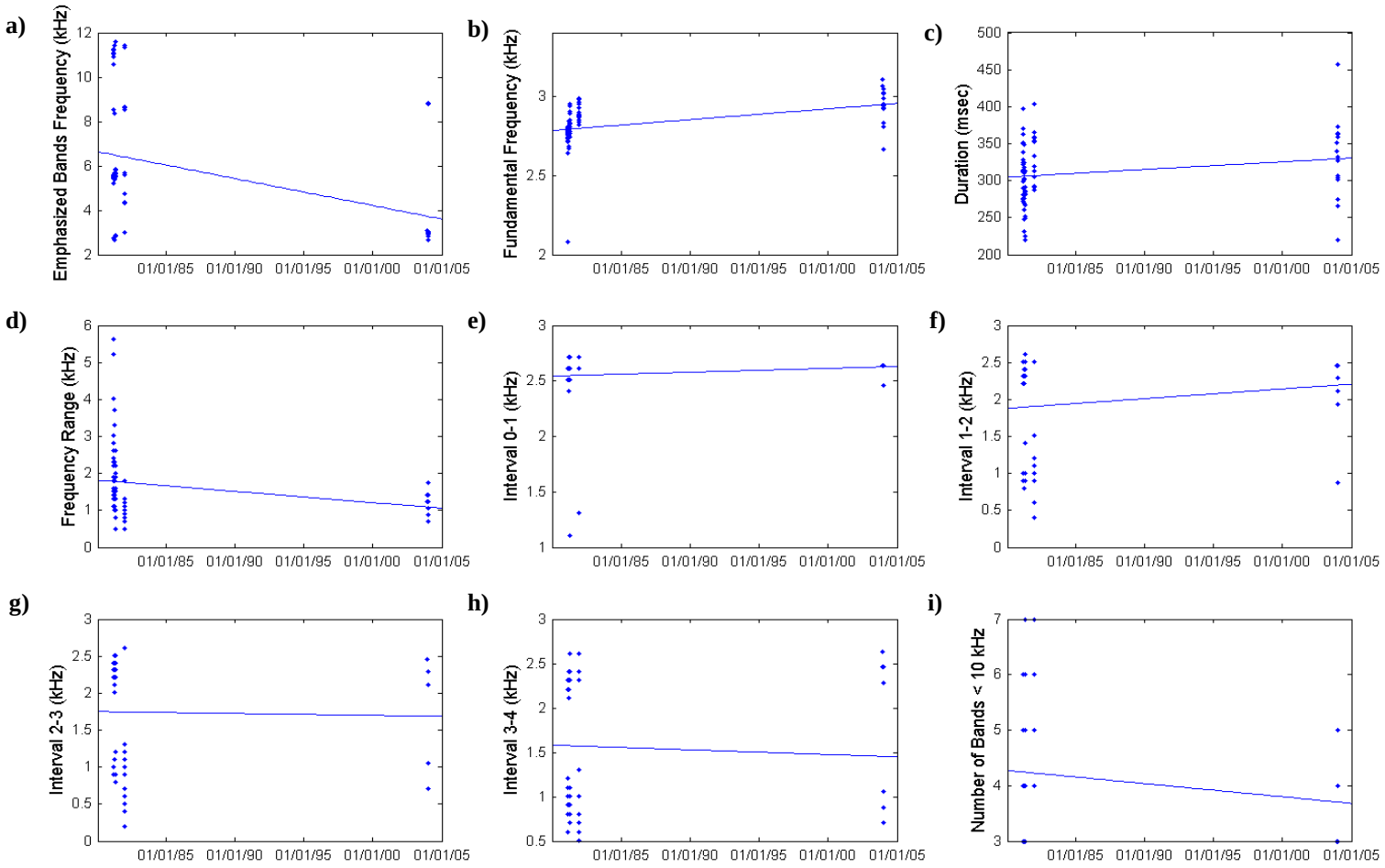

Figure 7. Plots of recording date versus acoustic parameter for Robin's vocalizations for every day he was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

adult both in the 1980's and 2002-2004), because he was only recorded on two separate days (December 18, 1980 and November 8, 2002). However, Wilcoxon signed-rank tests showed that frequency of the emphasized band, duration, interval 2-3 and interval 3-4 all significantly decreased over that time period, whereas frequency range and the number of bands less than 10 kHz significantly increased (Table 6 and Figure 8).

The second category of animals included those animals recorded when they were less than one year old and at least once more when they were between one and three years old (Janice, Lucille, Macon and Robin; Table 7, Figures 9-12). Four parameters of Janice's vocalizations significantly decreased between January 26, 2003 and December 31, 2003: frequency of emphasized band, fundamental frequency, interval 0-1, and interval 3-4 (Table 7 and Figure 9). Fundamental frequency of Lucille's vocalizations significantly increased between December 16, 1980 and April 21, 1983 (Table 7 and Figure 10). Three parameters of Macon's vocalizations did not significantly change between January 18, 2003 and December 31, 2003 (Table 7 and Figure 11), and three parameters of Robin's vocalizations did not change significantly between February 23, 1981 and December 10, 1981 (Table 7 and Figure 12).

Finally, the third category included adults recorded in at least two separate field seasons over a short (1-3 year) time period (Judith, June, Lucille, and Luna; Table 8, Figures 13-16). Duration, frequency range, and interval 1-2 of Judith's vocalizations significantly increased between January 19, 2003 and December 27, 2003 (Table 8 and Figure 13). Five parameters in June's vocalizations did not significantly change between December 22, 2002 and December 30, 2003 (Table 8 and Figure 14). Fundamental frequency, frequency range, and interval 0-1 of Lucille's vocalizations significantly decreased between January 25, 2003 and January 1, 2004 (Table 8 and Figure 15). Five parameters of Luna's vocalizations did not change between

Table 6. *p* values from Wilcoxon signed-rank tests on individual parameters for Gene, the only adult recorded in the 1980's and rerecorded in the 2000's.

	Emphasized Band	Fundamental Frequency	Duration	Frequency Range	# of Bands <10 kHz	Interval 0-1	Interval 1-2	Interval 2-3	Interval 3-4
Gene	0.0002	0.1000	0.0348	0.0556	0.0104	0.0694	0.0697	0.0097	0.0010

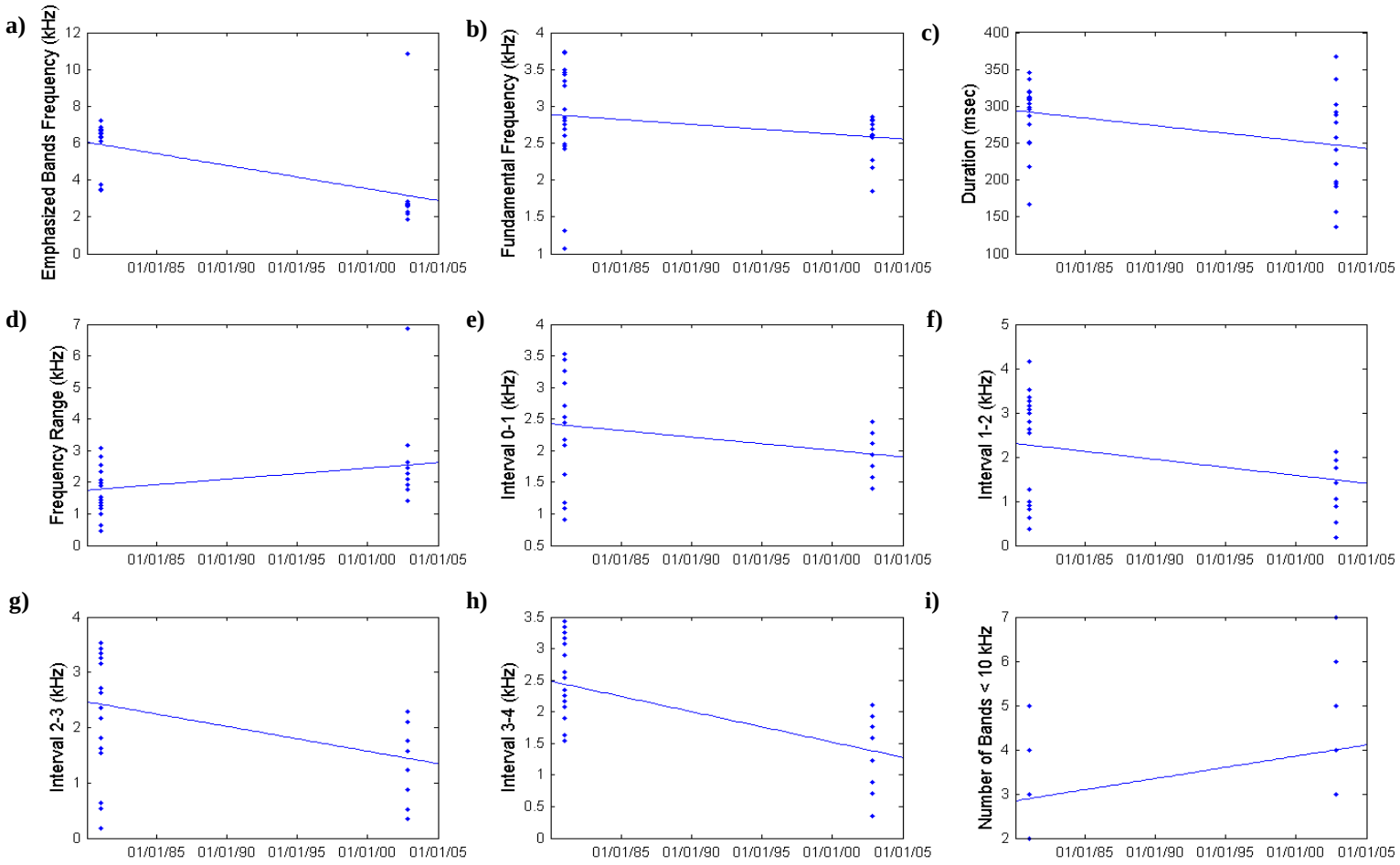

Figure 8. Plots of recording date versus acoustic parameter for Gene's vocalizations for every day he was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

Table 7. *p* values from one-way, repeated measures ANOVAs on vocal parameters of individual manatees recorded when they were less than one year old and at least once more when they were between one and three years old. Cases for which there were not enough distinct observations or in which data could not be normalized are marked with a "*". In some cases, not all parameters could be measured for each vocalization (i.e., if there were less than four bands), which resulted in different n values for different parameters.

Manatee	Emphasized Band	Fundamental Frequency	Duration	Frequency Range	# of Bands <10 kHz	Interval 0-1	Interval 1-2	Interval 2-3	Interval 3-4
Janice									
p	0.0004	0.0005	0.0701	*	*	0.0054	*	*	0.0014
F	114.16	107.32	6.02			30.11			61.14
num df	1	1	1			1			1
den df	4	4	4			4			4
n	45	45	45			45			45
Lucille									
p	*	0.0186	0.3871	0.5648	*	0.0801	*	*	0.1724
F		7.42	1.09	0.62		3.70			2.28
num df		2	2	2		2			2
den df		7	7	7		7			7
n		107	107	107		90			90
Macon									
p	*	0.7834	0.8073	0.3445	*	*	*	*	*
F		0.10	0.08	1.51					
num df		1	1	1					
den df		2	2	2					
n		48	48	48					
Robin									
p	*	0.1686	0.2175	0.2443	*	*	*	*	*
F		3.26	2.42	2.09					
num df		1	1	1					
den df		3	3	3					
n		60	60	60					

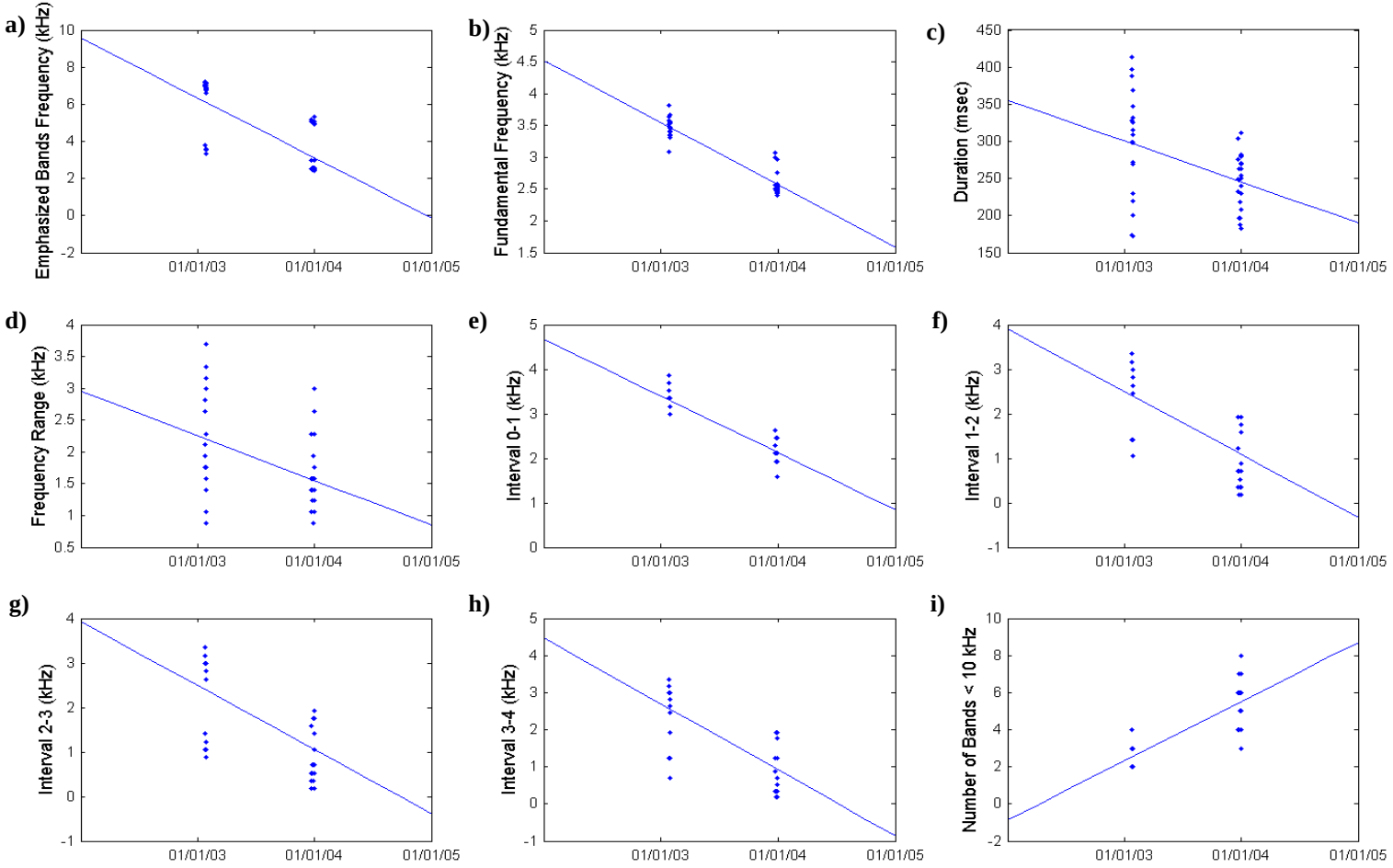

Figure 9. Plots of recording date versus acoustic parameter for Janice's vocalizations for every day she was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

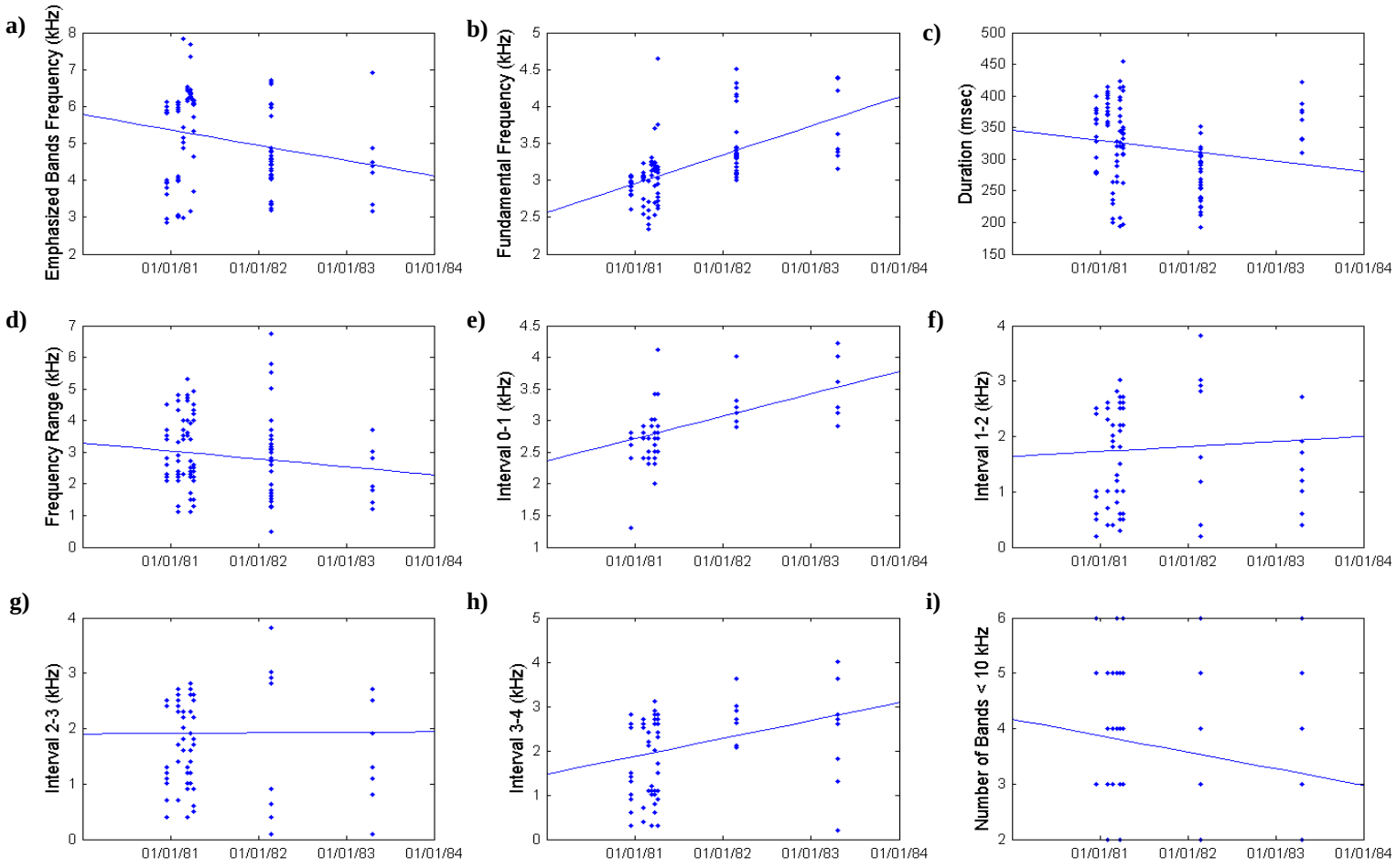

Figure 10. Plots of recording date versus acoustic parameter for Lucille's vocalizations for every day she was recorded as a calf showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

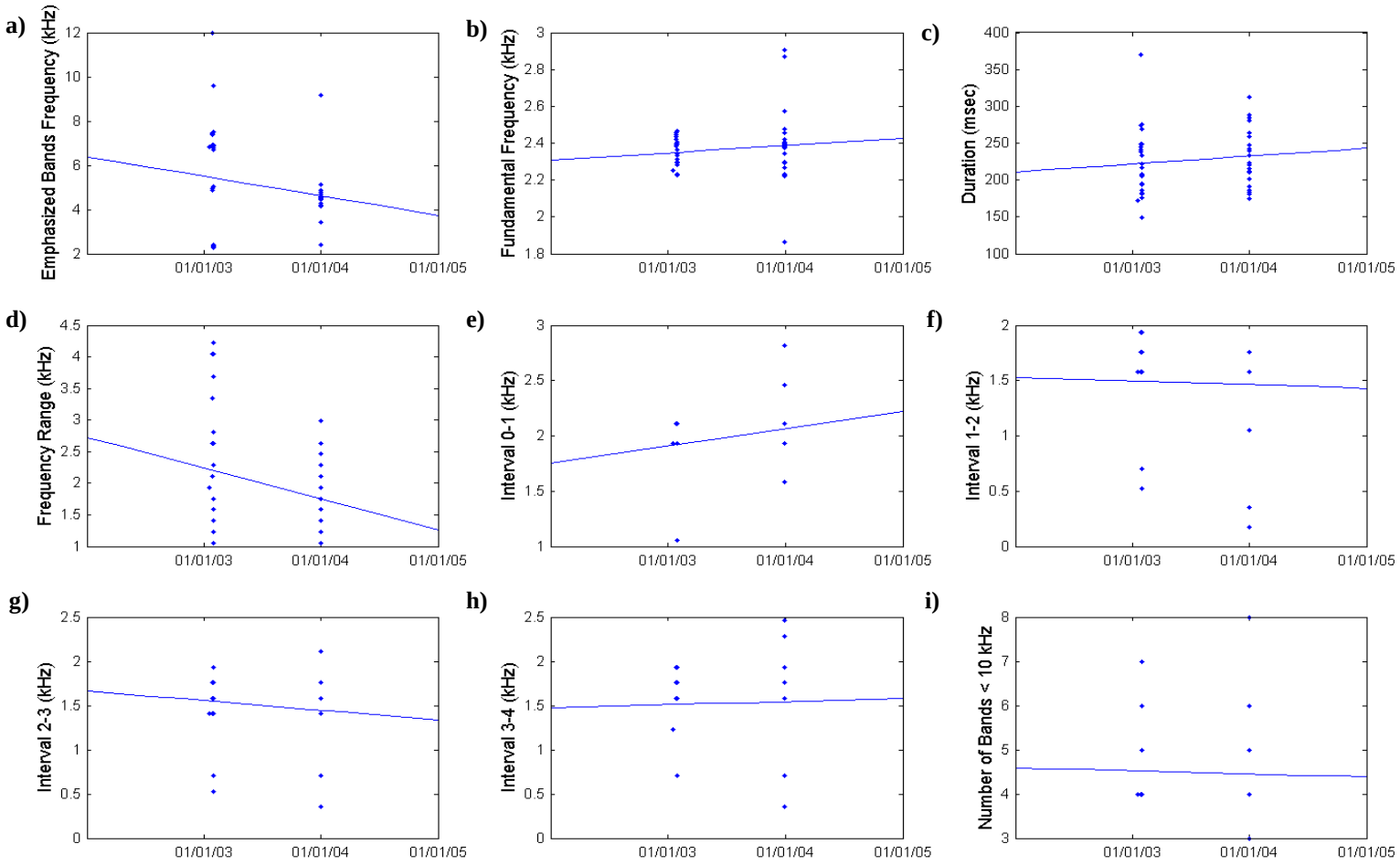

Figure 11. Plots of recording date versus acoustic parameter for Macon's vocalizations for every day she was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

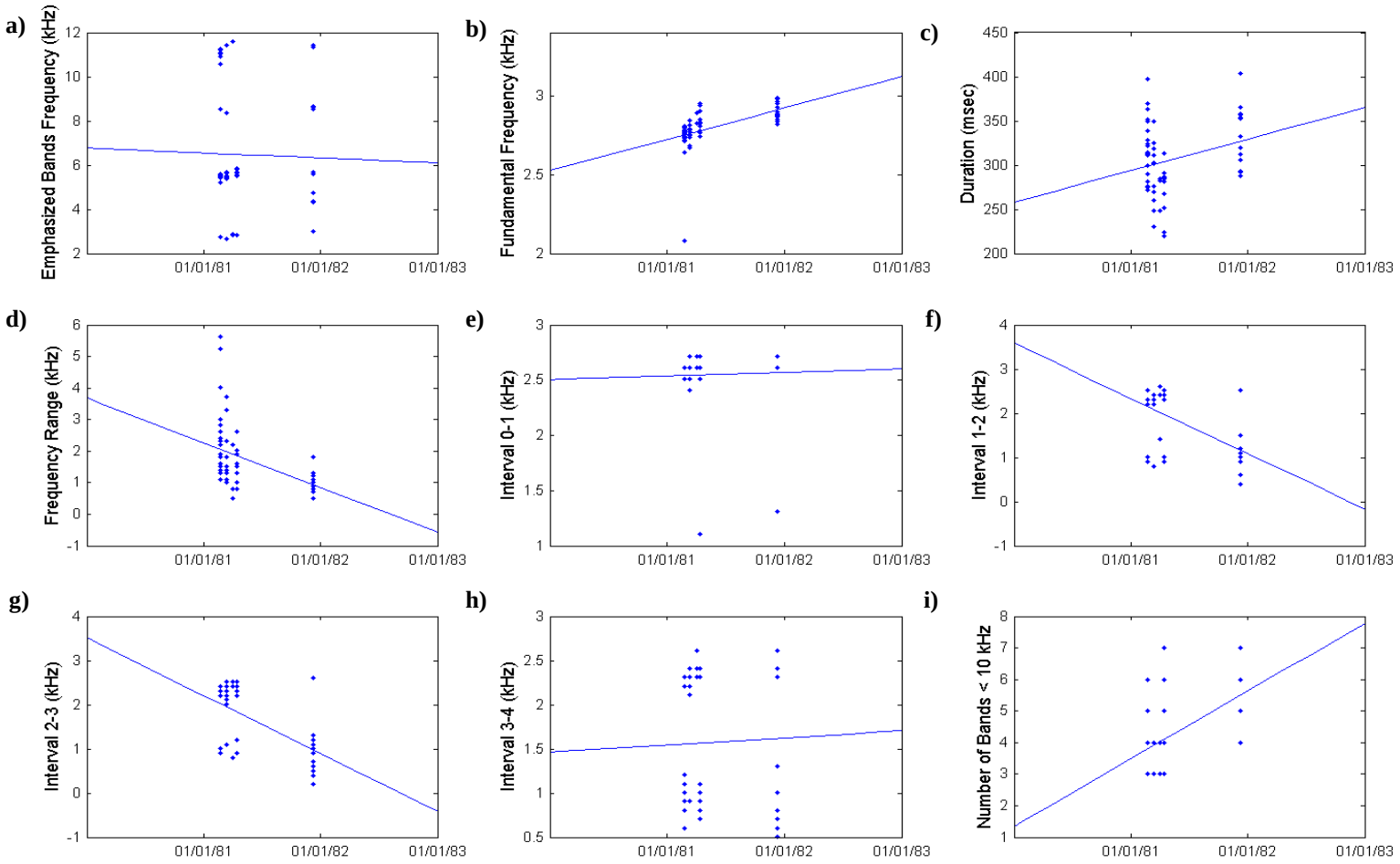

Figure 12. Plots of recording date versus acoustic parameter for Robin's vocalizations for every day he was recorded as a calf showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

Table 8. *p* values from one-way, repeated measures ANOVAs on vocal parameters of individual manatees recorded as adults in two separate field seasons. Cases for which there were not enough distinct observations or in which data could not be normalized are marked with a "*". In some cases, not all parameters could be measured for each vocalization (i.e., if there were less than four bands), which resulted in different n values for different parameters.

Manatee	Emphasized Band	Fundamental Frequency	Duration	Frequency Range	# of Bands <10 kHz	Interval 0-1	Interval 1-2	Interval 2-3	Interval 3-4
Judith									
p	0.1974	0.1974	0.0254	0.0013	0.0950	*	0.0010	0.6147	0.4820
F	2.38	2.38	12.08	64.38	4.74		75.42	0.30	0.60
num df	1	1	1	1	1		1	1	1
den df	4	4	4	4	4		4	4	4
n	31	31	31	31	31		31	31	31
June									
p	*	0.6258	0.2187	0.5782	*	0.1776	*	*	0.2805
F		0.29	2.41	0.39		3.08			1.72
num df		1	1	1		1			1
den df		3	3	3		3			3
n		23	23	23		23			23
Lucille									
p	0.0782	0.0491	0.7691	0.0278	*	0.0303	*	0.9527	0.0732
F	6.93	10.28	0.10	16.10		15.08		0.00	7.34
num df	1	1	1	1		1		1	1
den df	3	3	3	3		3		3	3
n	82	82	82	82		80		80	80
Luna									
p	*	0.4207	0.0659	0.8015	*	0.8725	0.9328	*	*
F		1.08	5.79	0.23		0.14	0.07		
num df		2	2	2		2	2		
den df		4	4	4		3	3		
n		32	32	32		16	16		

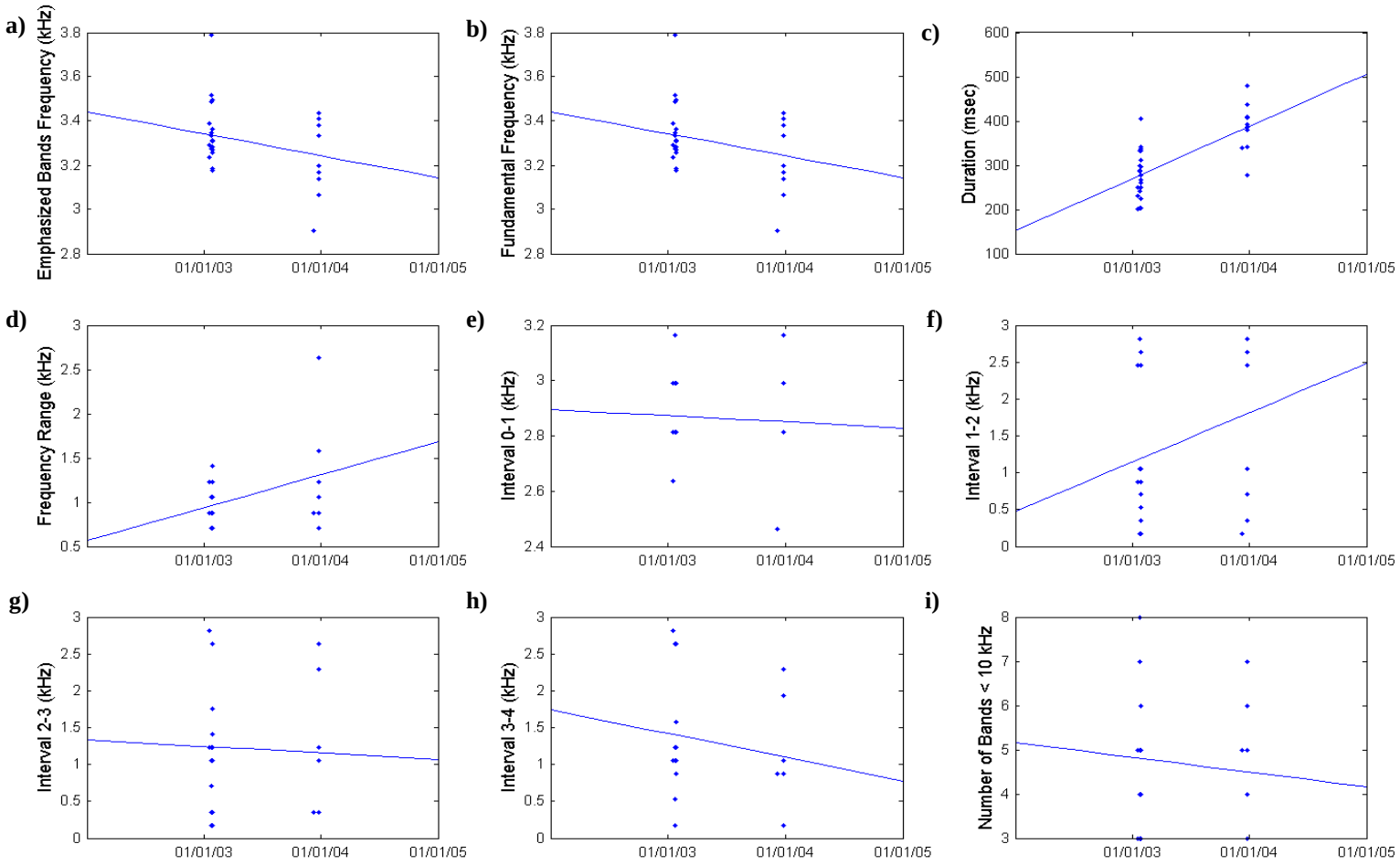

Figure 13. Plots of recording date versus acoustic parameter for Judith's vocalizations for every day she was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

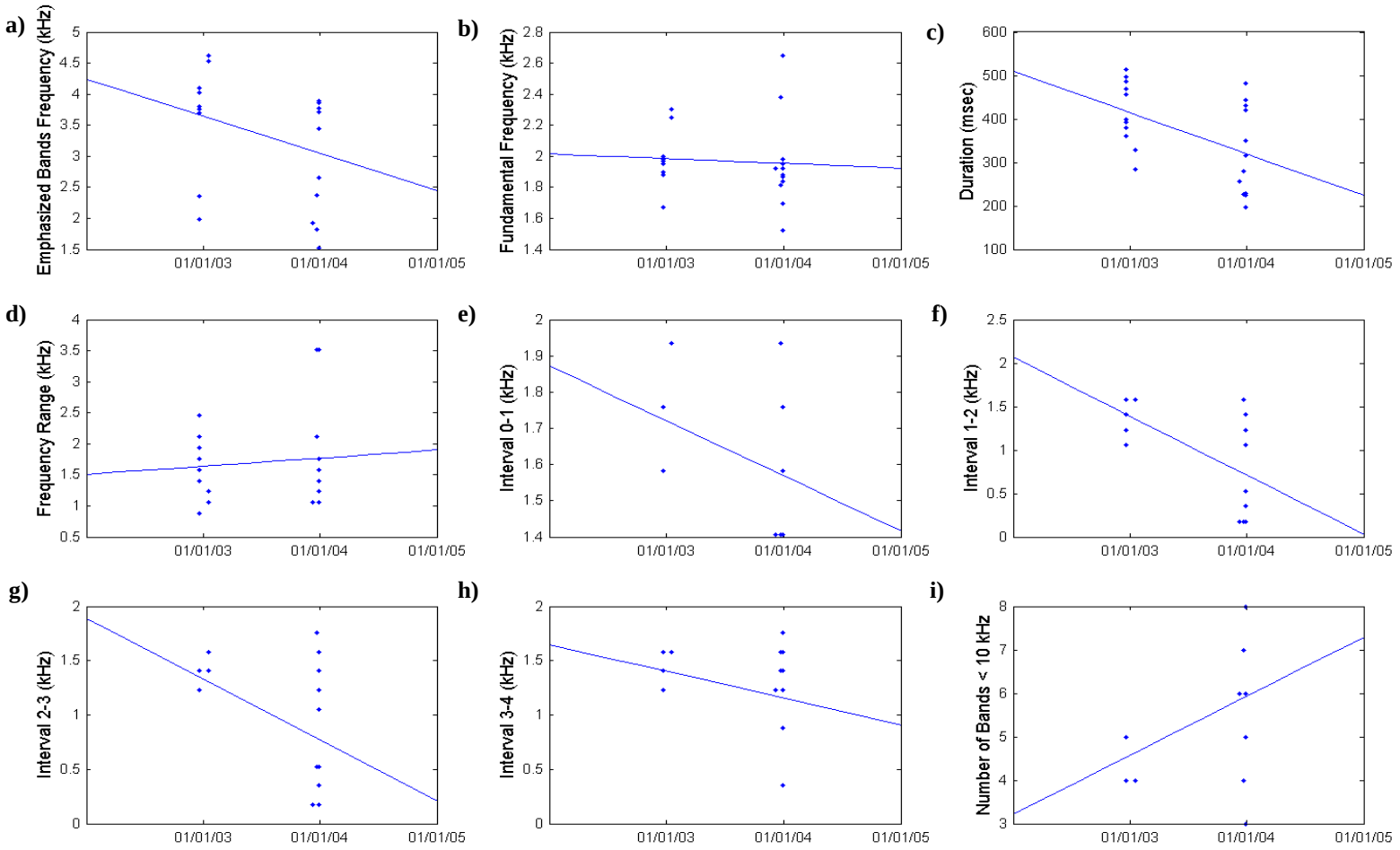

Figure 14. Plots of recording date versus acoustic parameter for June's vocalizations for every day she was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

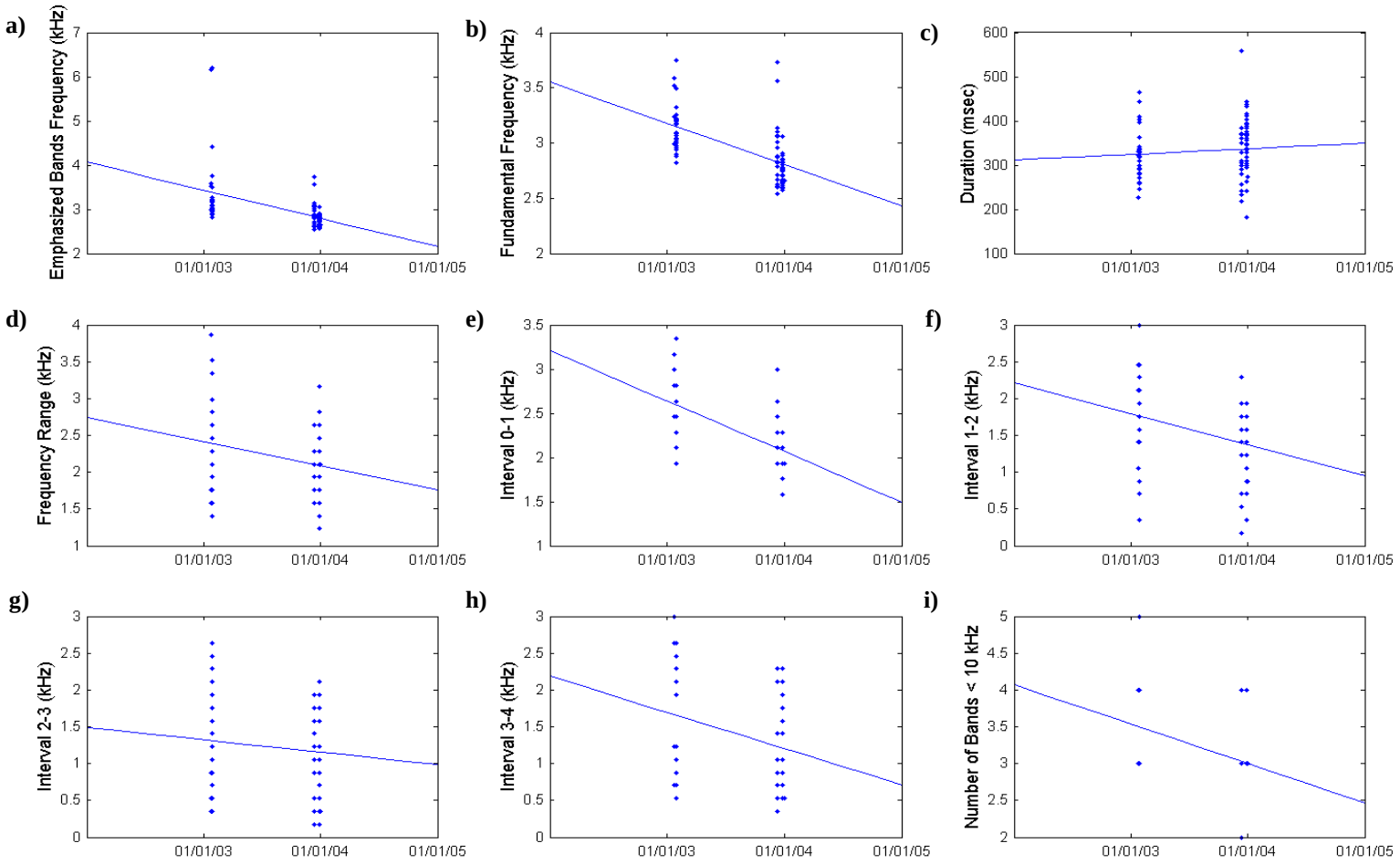

Figure 15. Plots of recording date versus acoustic parameter for Lucille's vocalizations for every day she was recorded as an adult showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

February 4, 1981 and April 21, 1983 (Table 8 and Figure 16).

The CVLDA on vocalizations of adults versus calves resulted in greater percentages than chance assigned correctly both to adults (82.2% of 506 vocalizations) and to calves (79.5% of 503 vocalizations). The CVLDA on vocalizations of female versus male calves resulted in greater percentages than chance assigned correctly both to female calves (66.0% of 250 vocalizations) and to male calves (58.1% of 253 vocalizations). For each of these analyses, there was one discriminant function (equal to one less than the number of groups), the most important parameter of which is listed in Table 4.

In comparison to adults (for those parameters that could be measured), calves had significantly higher emphasized band frequencies and significantly larger frequency ranges, intervals 0-1, and intervals 3-4 (Table 9, Figure 17). There were no other significant differences between calves and adults (Table 9). There were no significant differences between male and female calves for the four parameters that could be tested (Table 9, Figure 18).

The generalized linear model of vocalization rates during certain behaviors used 33 hours and 45 minutes of observations from 57 group follows. Vocalization rates are summarized in Table 10. Group size, which ranged from one individual to 25, did not significantly affect vocalization rates in any behavioral category; thus, call rates per minute could be averaged across group sizes. When looking at call rates per minute per manatee, manatees that were playing had significantly lower vocalization rates than those participating in any other behavior; manatees that were milling, socializing, surface resting, and traveling had significantly higher vocalization rates than those that were bottom resting; and manatees that were surface resting had significantly higher vocalization rates than those that were feeding (Table 11). When looking at calls rates per minute averaged across group sizes, manatees that were milling,

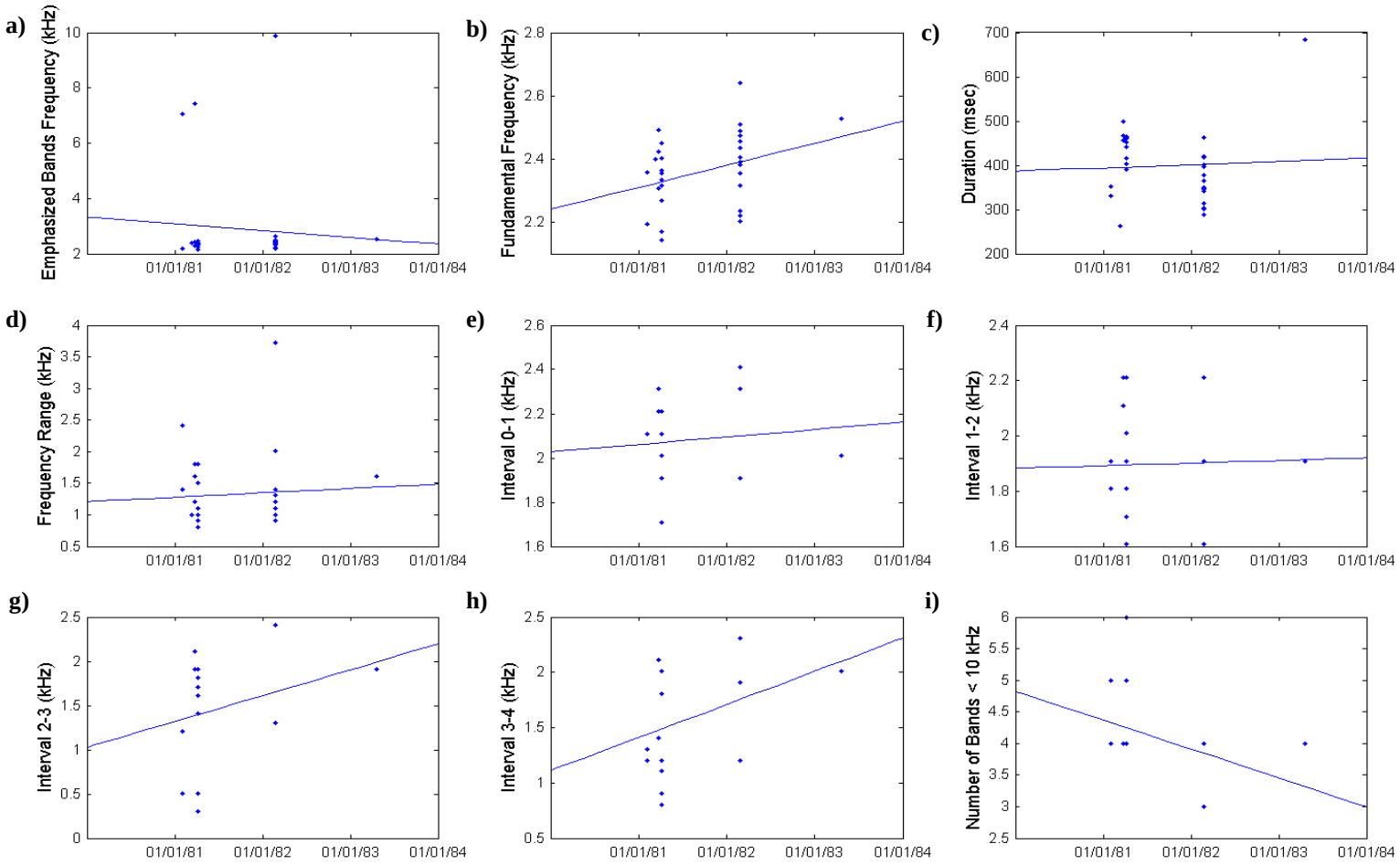

Figure 16. Plots of recording date versus acoustic parameter for Luna's vocalizations for every day she was recorded showing a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands < 10 kHz.

Table 9. *p* values from one-way ANOVAs performed on individual parameters for age classes (calves and adults) and sexes of calves (male and female). Cases for which data could not be normalized are marked with a "*". In some cases, not all parameters could be measured for each vocalization (i.e., if there were less than four bands), which resulted in different n values for different parameters.

	Emphasized Band	Fundamental Frequency	Duration	Frequency Range	# of Bands <10 kHz	Interval 0-1	Interval 1-2	Interval 2-3	Interval 3-4
Calves vs. Adults									
p	0.0054	0.1603	0.0739	0.0139	*	0.0150	*	*	0.0347
F	183.82	4.78	12.05	70.52		65.35			27.30
num df	1	1	1	1		1			1
den df	2	2	2	2		2			2
n	1067	1067	1067	1067		1021			1009
Male vs. Female Calves									
p	0.1427	0.7161	0.6365	0.8750	*	*	*	*	*
F	2.43	0.14	0.23	0.03					
num df	1	1	1	1					
den df	13	13	13	13					
n	521	521	521	521					

Figure 17. Box plots comparing all parameters between adults and calves: a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands less than 10 kHz.

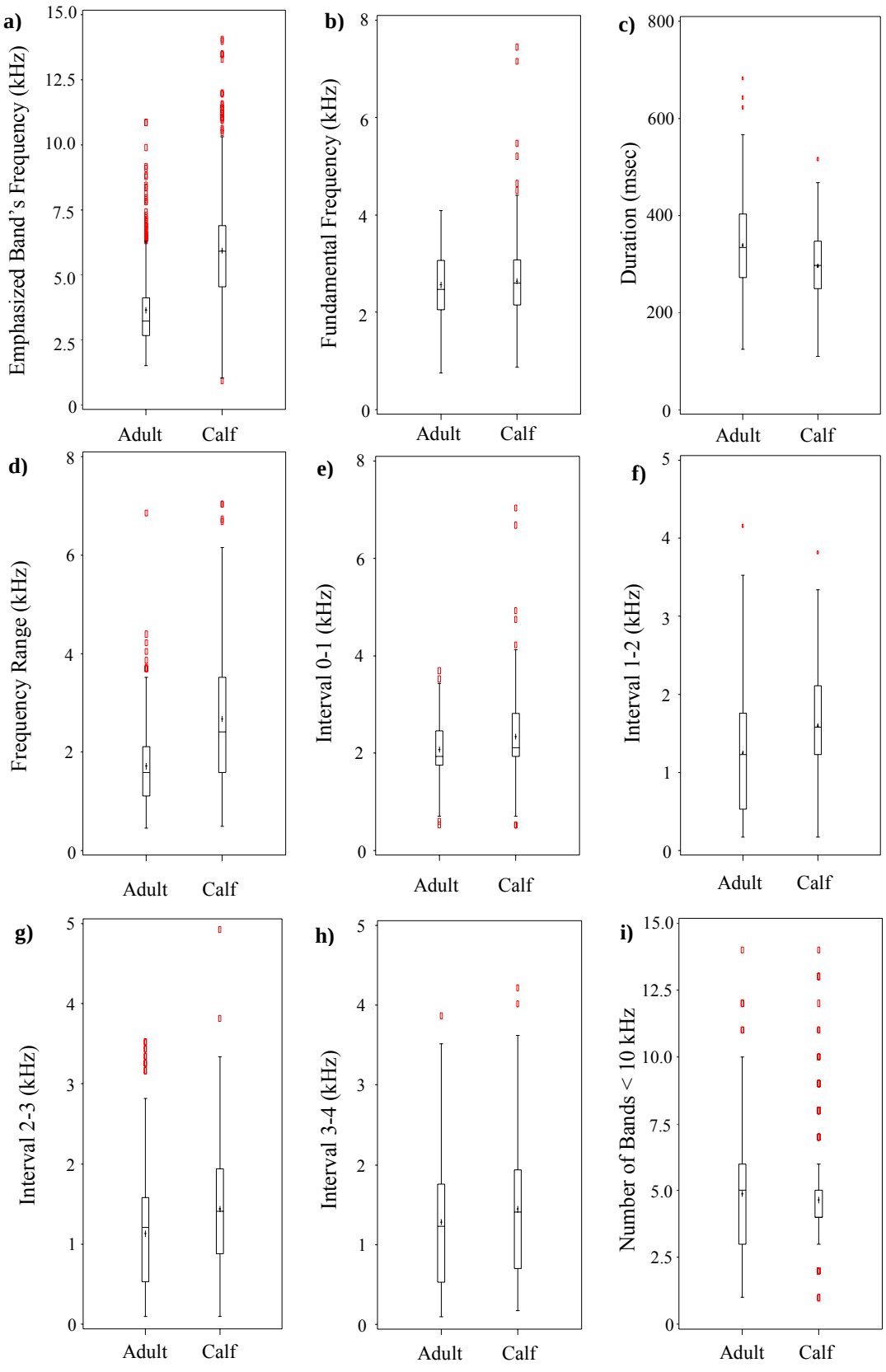
a)
Emphasized Band's Frequency (kHz)
15.0
12.5
10.0
7.5
5.0
2.5
0
Adult
Calf
b)
Fundamental Frequency (kHz)
8
6
4
2
0
Adult
Calf
c)
Duration (msec)
800
600
400
200
0
Adult
Calf
d)
Frequency Range (kHz)
8
6
4
2
0
Adult
Calf
e)
Interval 0-1 (kHz)
8
6
4
2
0
Adult
Calf
f)
Interval 1-2 (kHz)
5
4
3
2
1
0
Adult
Calf
g)
Interval 2-3 (kHz)
5
4
3
2
1
0
Adult
Calf
h)
Interval 3-4 (kHz)
5
4
3
2
1
0
Adult
Calf
i)
Number of Bands < 10 kHz
15.0
12.5
10.0
7.5
5.0
2.5
0
Adult
Calf

Figure 18. Box plots comparing all parameters between female and male calves: a) emphasized band's frequency (kHz), b) fundamental frequency (kHz), c) duration (msec), d) frequency range (kHz), e) interval 0-1 (kHz), f) interval 1-2 (kHz), g) interval 2-3 (kHz), h) interval 3-4 (kHz), and i) number of bands less than 10 kHz.

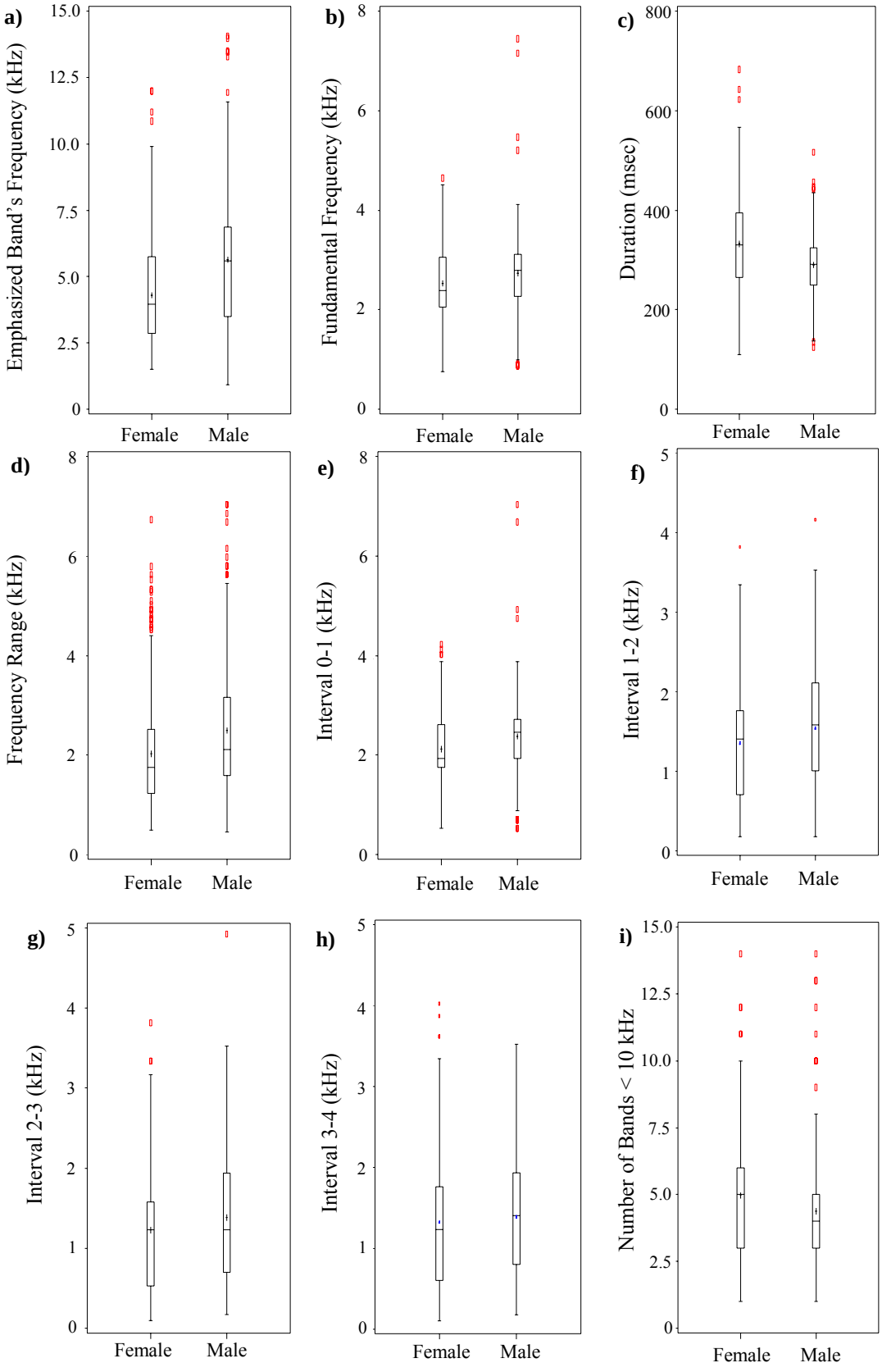
a)
Emphasized Band's Frequency (kHz)
15.0
12.5
10.0
7.5
5.0
2.5
0
Female
Male
b)
Fundamental Frequency (kHz)
8
6
4
2
0
Female
Male
c)
Duration (msec)
800
600
400
200
0
Female
Male
d)
Frequency Range (kHz)
8
6
4
2
0
Female
Male
e)
Interval 0-1 (kHz)
8
6
4
2
0
Female
Male
f)
Interval 1-2 (kHz)
5
4
3
2
1
0
Female
Male
g)
Interval 2-3 (kHz)
5
4
3
2
1
0
Female
Male
h)
Interval 3-4 (kHz)
5
4
3
2
1
0
Female
Male
i)
Number of Bands < 10 kHz
15.0
12.5
10.0
7.5
5.0
2.5
0
Female
Male

Table 10. Vocalization rates for eight different behaviors obtained using the generalized linear model on 33 hours and 45 minutes of observations from 57 group follows. All vocalizations regardless of type were used.

Behavior	# of calls/minute/manatee	Mean # of calls/minute averaged across group sizes
Bottom Rest	0.11	1.55
Feed	0.11	1.88
Mill	0.21	2.73
Play	0.02	1.71
Social	0.22	3.81
Surface Rest	0.32	2.43
Travel	0.19	2.15
With Boat	0.19	1.62

Table 11. Results from the generalized linear model conveying significant differences between vocalization rates when looking at rates per minute per manatee (with $\alpha < 0.10$ significant). All vocalizations regardless of type were used.

Results	*p* value
Bottom Rest > Play	< 0.0001
Feed > Play	0.000007
Mill > Bottom Rest	< 0.0001
Mill > Play	< 0.0001
Social > Bottom Rest	0.004
Social > Play	< 0.0001
Surface Rest > Bottom Rest	0.0001
Surface Rest > Feed	0.019
Surface Rest > Play	< 0.0001
Travel > Bottom Rest	0.020
Travel > Play	< 0.0001
With Boat > Play	0.001

socializing, and surface resting had significantly higher vocalization rates than those that were bottom resting; manatees milling had significantly higher vocalization rates than those with boat; and manatees socializing had significantly higher vocalization rates than those playing and with boat (Table 12). There were no other significant differences in vocalization rates among behavioral categories. The percentage of focal-follow minutes for each group size is listed in Table 13. The total number of manatee minutes for each behavioral category is shown in Table 14.

DISCUSSION

Individually Distinctive Vocalizations

Results of this study support the hypothesis that Florida manatee vocalizations have individually distinctive features. The CVLDA assigned a higher percentage of calls to the correct caller than expected by chance in all but three cases. In many cases, a much higher percentage was assigned than expected by chance (19 manatees had 50% or more of their calls correctly classified; Table 2). Misclassifications may have been due to several different factors. First, it is possible that while making recordings in the field, I misidentified which manatee was vocalizing and attributed a call to a manatee when it was in fact from another individual. Secondly, I used all calls from an individual unless the calls were noisy. Thus, if manatees have context-specific call types, or if they alter parameters of their calls depending on motivational state (neither of which were addressed in this study), then lower percentages of calls would likely be correctly classified to each individual. The high classification accuracy (89.9-97.3%) of calls of common marmosets could have resulted from the use of only one call type, the phee call

Table 12. Results from the generalized linear model conveying significant differences between vocalization rates when looking at rates per minute averaged across group sizes (with $\alpha < 0.10$ significant). All vocalizations regardless of type were used.

Results	*p* value
Mill > Bottom Rest	< 0.0001
Mill > With Boat	0.008
Social > Bottom Rest	0.077
Social > Play	0.035
Social > With Boat	0.060
Surface Rest > Bottom Rest	0.077

Table 13. Percentage of focal-follow minutes for each group size during behavioral observations.

Group size (# of manatees)	% of focal-follow minutes
1	55.9%
2	14.5%
3	13.0%
4	6.0%
5	4.1%
6	1.8%
7	1.9%
8	0.9%
9	0.6%
10	0.3%
11	0.2%
12	0.2%
13	0.1%
25	0.6%

Table 14. Total number of manatee minutes spent in each behavioral category calculated by summing the number of minutes that each individual manatee spent in a particular behavior.

Behavioral Category	# of manatee minutes
Bottom Rest	1756
Feed	253
Mill	982
Play	18
Social	217
Surface Rest	16
Travel	449
With Boat	9

(Jones *et al.* 1993). It is unknown if manatees have discrete call types; however, if this were found to be the case, then it is likely that a higher classification accuracy would result if only one call type was examined.

Another factor that may have influenced misclassifications was that the interval measurements were often highly correlated (except for interval 0-1, which was usually larger than the others). In 552 of 1058 calls (52.2%), intervals 1-2, 2-3, and 3-4 were highly correlated, and in some cases, interval 0-1 was also correlated. In the remaining 47.8% of calls, the intervals were not correlated due to the presence of subharmonics. Thus, in about half of the vocalizations, the multiple interval measurements were not providing any additional information about the vocalizations' distinctiveness. I found that fundamental frequency, interval 0-1, and the emphasized band's frequency range were all important in discriminating among calls (Table 4), although other studies have found different parameters to be important. For identifying individual Antillean manatee vocalizations, center frequency of the first band was an important parameter (Alicea-Pou 2001); for identifying individual Amazonian manatee vocalizations, maximum and minimum fundamental frequencies were important parameters (Sousa-Lima *et al.* 2002). Contour, or the overall shape of the vocalization, may also contribute to individual variability in manatee vocalizations, as found by Sousa-Lima and da Silva (2001) in Amazonian manatees. Such is the case for bottlenose dolphins, which produce signature whistles with very distinctive contours (Caldwell and Caldwell 1965). For example, even though 68.8% of Lucille's calls were correctly classified when using only the 2002-2004 vocalizations, this percentage probably would have increased if contour had been taken into account, since she made an unusual "trill" (undulations in frequency) which was not included in the nine parameters measured. Although several of the manatees' vocalizations in this study had similar contours,

there were subtle differences—differences that could convey important signature information (Figure 4). Future analyses should utilize a program such as ACOUSTAT (Fristrup and Watkins 1995), which measures 97 attributes of a signal including characteristics of its contour.

Other parameters, or combinations of parameters, may also be important in manatee calls, such as subharmonics. This study only accounted for subharmonics if they were 50% or greater of the call's duration; however, there were many subharmonics that were less than 50%. Wilden *et al.* (1998) stated that biphonation, subharmonics, and chaos regularly appear in vocalizations of many mammals and can be understood with nonlinear dynamics. Biphonation is when two separate fundamental frequencies appear that are independent of one another. Subharmonics appear in non-integer multiples (1/2 or 1/3 usually) of the fundamental frequency. Chaos can also be called noise, and does not necessarily have harmonics, although segments with subharmonics ("periodic windows") often interrupt chaotic segments (Wilden *et al.* 1998). Chaos mostly has been seen in neonate vocalizations [i.e., in African wild dogs (*Lycaon pictus*) and humans], perhaps due to a newborn's underdeveloped phonatory control (Wilden *et al.* 1998). Tokuda *et al.* (2002) stated that nonlinear analyses might be important to the study of animal vocalizations because, to date, noisy calls have been excluded from most research. Such nonlinear phenomena have been found in mammals such as humans, African wild dogs, macaques, and piglets, as well as in birds (Wilden *et al.* 1998, Tokuda *et al.* 2002). These nonlinear phenomena may occur because of desynchronization of vocal folds or of vibratory modes of one fold (Wilden *et al.* 1998). Wilden *et al.* (1998) stated that all three nonlinear phenomena may add to the enrichment of the acoustic repertoire and may be indicators of individuality, motivation, or status (age, sex, or physiological condition). Tooze *et al.* (1990) found that timber wolves had individually specific subharmonics in their howls (Wilden *et al.*

1998). I found subharmonics and chaos in Florida manatee vocalizations, as well as possible biphonation (although it is possible two manatees were vocalizing at the same time in the few cases found; Figure 19). Scooter, a captive, 6-12 month old orphan calf weighing 30.5 kg and extending 119 cm (the weight and length of a typical newborn), produced 208 out of 241 vocalizations (86%) that were chaotic without distinct bands (Figure 20). It is possible that his phonatory control was underdeveloped at the time of recording. All other manatees had calls with more harmonic structure, frequently with subharmonics and occasionally with chaotic segments. Anderson and Barclay (1995) found all three nonlinear phenomena in the vocalizations of dugongs (Wilden *et al.* 1998): spectrograms of some dugong vocalizations showed subharmonics, chaos was displayed in the barks, and biphonation was shown from one dugong that made a bark and a chirp-squeak simultaneously. Thus, it is possible that such nonlinear phenomena contribute to individual variability in Florida manatee vocalizations. Because it is currently not known how manatees produce vocalizations, the study of nonlinear phenomena may provide insight into this process as well.

Three manatees had lower percentages of their vocalizations assigned to them than expected by chance. One of these was Loverboy, a subadult who had been hit by a watercraft, causing lacerations to his head but no brain damage (personal communication from Lowry Park Zoo veterinarian, Dr. David Murphy 2005); his overall behavior was very atypical and unusual. His injury may have affected his vocal production, which then could have resulted in his 0% correct classification rate. Janice also was assigned a lower percentage of calls than expected by chance; she was recorded as a calf during one field season and then as a subadult the following field season. It appears that her vocalizations were either not fully developed at her first recording session and/or not stable between years, both of which would result in a low

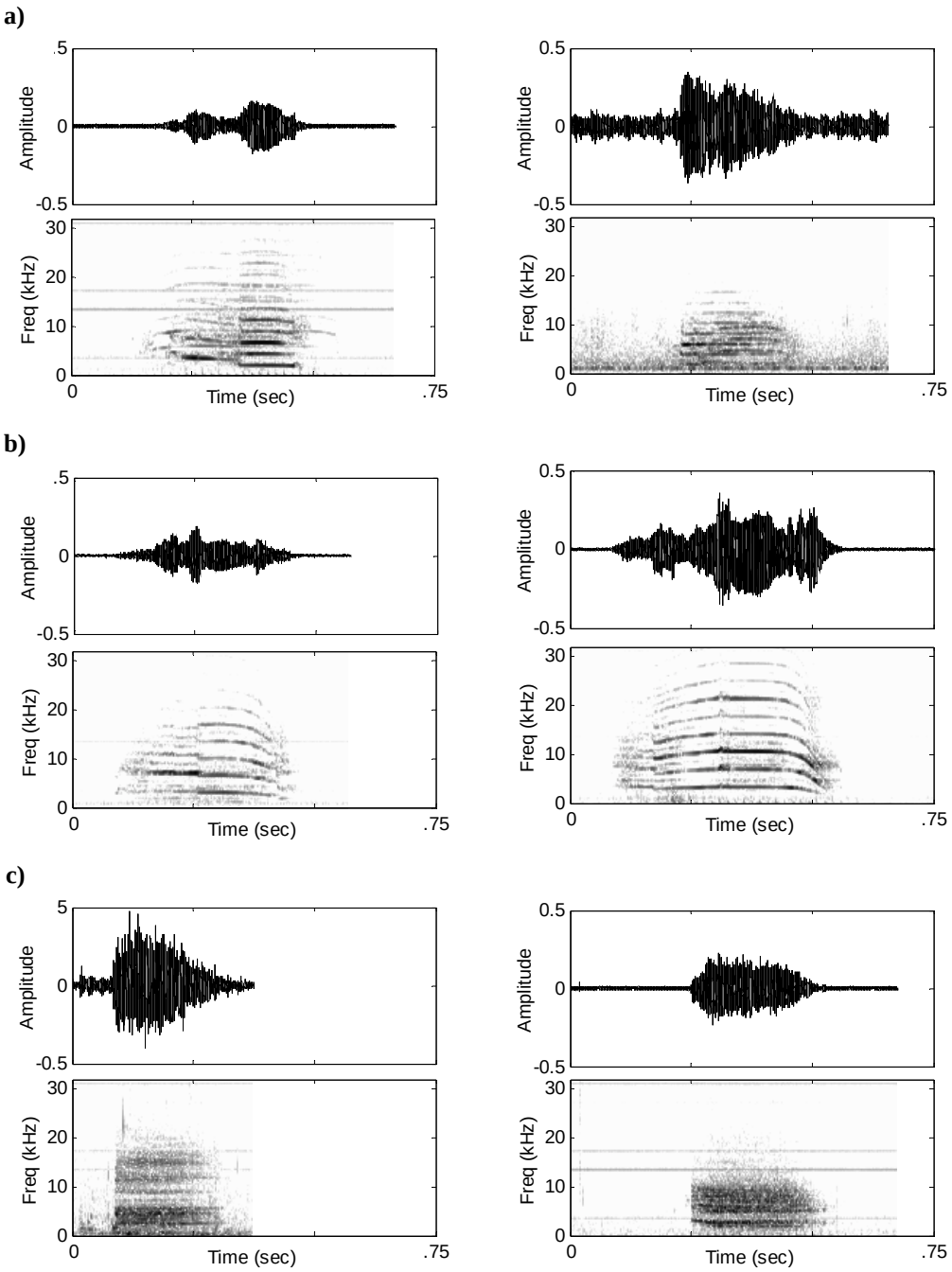

Figure 19. Spectrograms of calls showing a) possible biphonation, b) subharmonics, and c) chaos. Biphonation is when two separate fundamental frequencies appear that are independent of one another. Subharmonics appear in non-integer multiples of the fundamental frequency. Chaos refers to the noisy quality of a vocalization, which may or may not have bands.

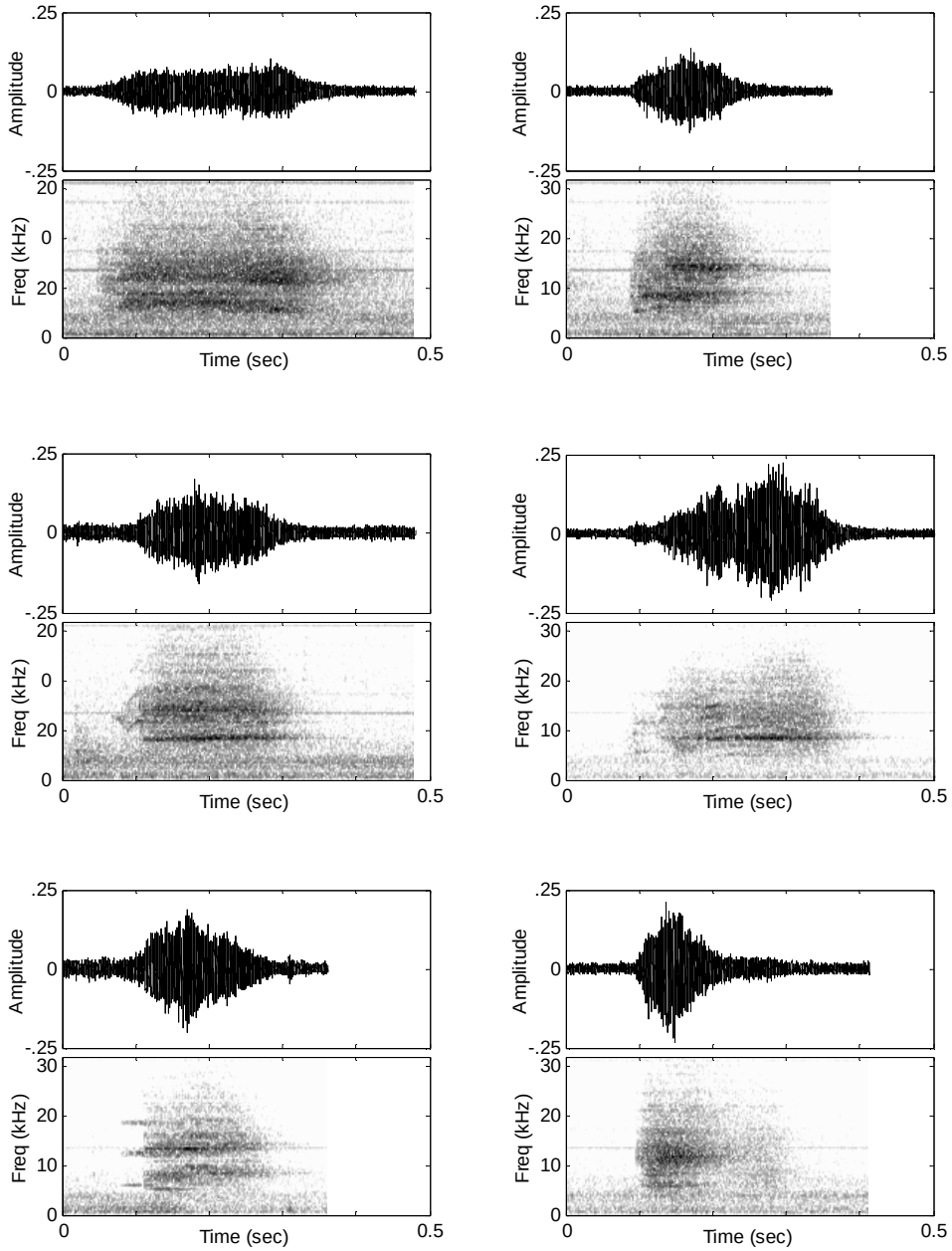

Figure 20. Spectrograms of six of Scooter's calls, showing chaos, defined as noise that may or may not have harmonics.

classification percentage. To examine these possibilities, CVLDAs were performed using calls from either the first or second field season in which she was recorded. When only using her first year calls, a much higher percentage of calls was correctly assigned to her than expected by chance (52.0%); the same was true when only calls from the second field season were used (55.0%). A similar analysis was undertaken for June's vocalizations; although her percentage of correctly classified calls was higher than expected by chance (13.0%), she had also been recorded over two field seasons. Higher percentages of calls were assigned to her when calls from only the first field season (54.6%) and from only the second field season (25.0%) were used.

The third individual that had a lower percentage of vocalizations assigned to her than expected by chance (Goodland, a subadult who had been brought to Lowry Park Zoo due to pneumothorax; personal communication with Dr. David Murphy 2005) had variable calls during the two days she was recorded. However, this does not mean that she does not have an individually distinctive vocalization. Bottlenose dolphins, which are known to have signature whistles, also produce variant whistles (Caldwell *et al.* 1990). In some instances, dolphins may produce more variant whistles than signature whistles (Janik and Slater 1998). Further research is needed to determine if such variability exists in the acoustic repertoire of manatees. It is also interesting to note that all three animals that had below chance percentages of calls correctly assigned to them were subadults during at least one recording session, and that they were the only subadults recorded in this study. Thus, it is possible that manatees in the subadult stage alter their call parameters or have variable calls. Thus, future research should examine stereotypy and stability of subadult vocalizations.

Overall, the results of this study add further support to previous research on individual distinctiveness in manatee vocalizations (O'Shea *et al.* 2000, Alicea-Pou 2001, Alicea-Pou *et al.* 2001, and Sousa-Lima *et al.* 2002). Just as Alicea-Pou (2001) could consistently identify certain manatees based on characteristic sound quality and pitch of their vocalizations, I was able to do the same with two individuals (Donna and Lucille) in the field. On three separate occasions, I was able to predict that Donna was in the area before visually sighting her. After hearing what I thought sounded like her call on these occasions, I looked around for her and found her each time. The same thing happened with Lucille (who had a very unique trill to her call that was also present when she was a calf 22 years earlier) on two occasions. I also noticed that the vocalizations of calves seemed to have a much higher pitch than those of adults. These anecdotal observations support the idea that manatees have individually distinctive vocalizations, and that age differences occur in their calls.

A CVLDA suggested that there are sex differences in manatee calls, because calls were assigned to the correct sex a higher percentage of time than expected by chance. ANOVAs could only be performed on four parameters, but none were significant (Table 9). It is possible that a combination of the nine parameters analyzed in this study may be important in making this distinction. Sousa-Lima *et al.* (2002) found specific and significant differences between the sexes in Amazonian manatees, with females having shorter durations but higher fundamental frequencies than males (Sousa-Lima *et al.* 2002).

Individual Recognition

Results indicating that Florida manatee vocalizations indeed show individual variation add to the first component necessary for documenting individual recognition (individual

variability in calls; Beer 1970). However, one issue that needs to be addressed is the amount of individual variability that is biologically significant versus statistically significant. For example, a 5% correct classification rate is statistically significant but would not likely be sufficient for individual recognition. However, 19 out of 33 manatees had 50% or more of their calls correctly classified, which suggests that the individual distinctiveness of manatee calls may be biologically significant. In addition, the integration of additional parameter measures (as discussed previously) may increase the reliability of these classifications, especially if different parameters, or combinations of parameters, may be important for determining individual distinctiveness in different animals.

Some evidence for the second component necessary for documenting individual recognition (observation of how vocalizations affect behavior; Beer 1970) was also provided by observations made while in the field making recordings. On two separate occasions, I observed one of Donna's twins bottom resting in a large group when Donna and her other twin started to leave. I heard a call or two that sounded similar to Donna's calls, after which the twin that was bottom resting almost immediately started heading in the direction of his mother. During this time, no other manatee changed its activity or responded to the call. Alicea-Pou (2001), O'Shea *et al.* (2000), and Hartman (1979) report similar anecdotal evidence for individual recognition. Future research should investigate this second component more quantitatively, as well as focus on the last component necessary for documenting individual recognition by conducting playback experiments to determine how animals react to sounds produced by different individuals (Beer 1970). This third component should probably be addressed before any additional analyses of the distinctiveness of vocalizations are carried out.

Stability and Changes in Vocalizations Over Time

Results do not support the hypothesis that individual Florida manatee vocalizations are stable over long time periods. Only one of the four animals (Donna) recorded in the 1980's and then again in 2002-2004 increased her percentage of correctly assigned calls when the CVLDA was performed using all of the data (Tables 2 and 3). The percentage of calls correctly assigned to the other three (Gene, Lucille, and Robin) decreased when their vocalizations from the 1980's were added, even though their percentages were still higher than expected by chance (Tables 2 and 3). Two of these three individuals (Lucille and Robin) were calves when they were first recorded in the 1980's; thus, it is possible that their calls underwent maturational changes. Results from the CVLDA of vocalizations of adults versus calves suggest that there are age differences, because calls were correctly assigned to age classes a higher percentage of time than expected by chance. In addition, of all misclassifications in the linear discriminant analyses that were greater than 20% (Tables 2-3), most were within the same age class and not across age classes, which also implies age class differences inherent in calls. Only two misclassifications in Table 2 were across age classes: Georgia (an adult) was misclassified as Fritz (a calf), and Goodland (a subadult) was misclassified as Calista (an adult). Similarly, only three misclassifications in Table 3 were across age classes: Georgia was once again misclassified as Fritz, Goodland was once again misclassified as Calista, and Gray (a calf) was misclassified as Lorelei (an adult). Each of these across-age-class misclassifications consisted of a wild animal and an animal that had been in captivity (Georgia was raised in captivity and later released, Goodland had been brought into captivity as a 2 ½ year old five months prior to recording, and Lorelei was born in captivity and has never been released). It is possible that the captive rearing of both Georgia and Lorelei may have had an effect on their vocal development, thus potentially

contributing to the misclassification of Georgia's calls as those of a calf and of a calf's calls as those of Lorelei. It is also possible that subadults and adults produce vocalizations with similar features, which could have contributed to the misclassifications for Goodland.

Results from ANOVAs also suggest that calls are not stable over long time periods. Donna, Lucille, and Robin had relatively high percentages of significant changes in their parameters (between 50-63%; Table 5). However, it should be noted once again that all three of these animals were calves when they were first recorded in the 1980's. Thus, maturation could account for the high percentage of changes seen. All three manatees recorded as calves and then as adults 19+ years later showed significant decreases in emphasized band frequency (Table 5 and Figures 5-7). This finding is consistent with the overall comparison of calf and adult vocalizations, where calves had significantly higher emphasized band frequencies than adults (Table 9 and Figure 17a). Lower emphasized band frequencies in adults are typical of most maturational changes seen in other species, where larger individuals produce lower frequency vocalizations than smaller individuals (Boughman and Moss 2003). Such a change could be advantageous, since lower frequencies travel longer distances in water and may facilitate mothers staying in contact with their calves. No specific pattern was seen in other parameters that these three animals changed. The duration of Donna's vocalizations significantly increased, which concurs with the findings of Sousa-Lima *et al.* (2002), who found that Amazonian manatees increase the duration of their calls with age. No other parameters of Donna's vocalizations changed significantly over time. The overall stability in Donna's vocal parameters concurs with the results of the CVLDAs, in which the percentage of Donna's calls that were correctly classified increased when data from all years were used (Tables 2-3).

For Lucille, five parameters (including emphasized band frequency) changed significantly between 1980 and 2003, explaining the decrease in the percentage of her calls that were correctly classified in the linear discriminant analyses when data from all years were used (Tables 2-3). As a calf, Lucille produced vocalizations with different contours. One was a simple contour with little modulation that a majority of calves in this study produced (Figure 21c, e, and k, for example), while another had more frequency modulation (Figure 21a, b, and j, for example). The calls with greater frequency modulation also varied in the degree of modulation; those calls with more frequency modulation sounded like a "trill" and were similar both visually and aurally to vocalizations she produced in 2002-2004 (Figure 21d, f, o, and p, for example). It may be that in 1980-1983 she was still developing her individually distinctive vocalization. This seems likely given that four of five parameters that significantly changed in her vocalizations were the same four parameters that were significantly different for calves versus adults (Tables 5 and 9): emphasized band, frequency range, interval 0-1, and interval 3-4. Thus, it is possible that Lucille was going through maturational changes that Donna had already completed by the time she was first recorded. Unfortunately, nothing is known about the process of vocal development in manatees. Vocal development in bottlenose dolphins is highly variable, taking anywhere from 1 ½ months to over 17 ¼ months (at which time one male calf still had not developed a signature whistle; Caldwell and Caldwell 1979). It is possible that similar variability in the timing of vocal development occurs in manatees; Lucille may have undergone a prolonged vocal development phase whereas Donna may have solidified her call early in life. Future studies should look into the time course of development of vocalizations in manatees.

In Robin's vocalizations, emphasized band frequency was significantly higher and fundamental frequency was significantly lower as a calf than as an adult. Although fundamental

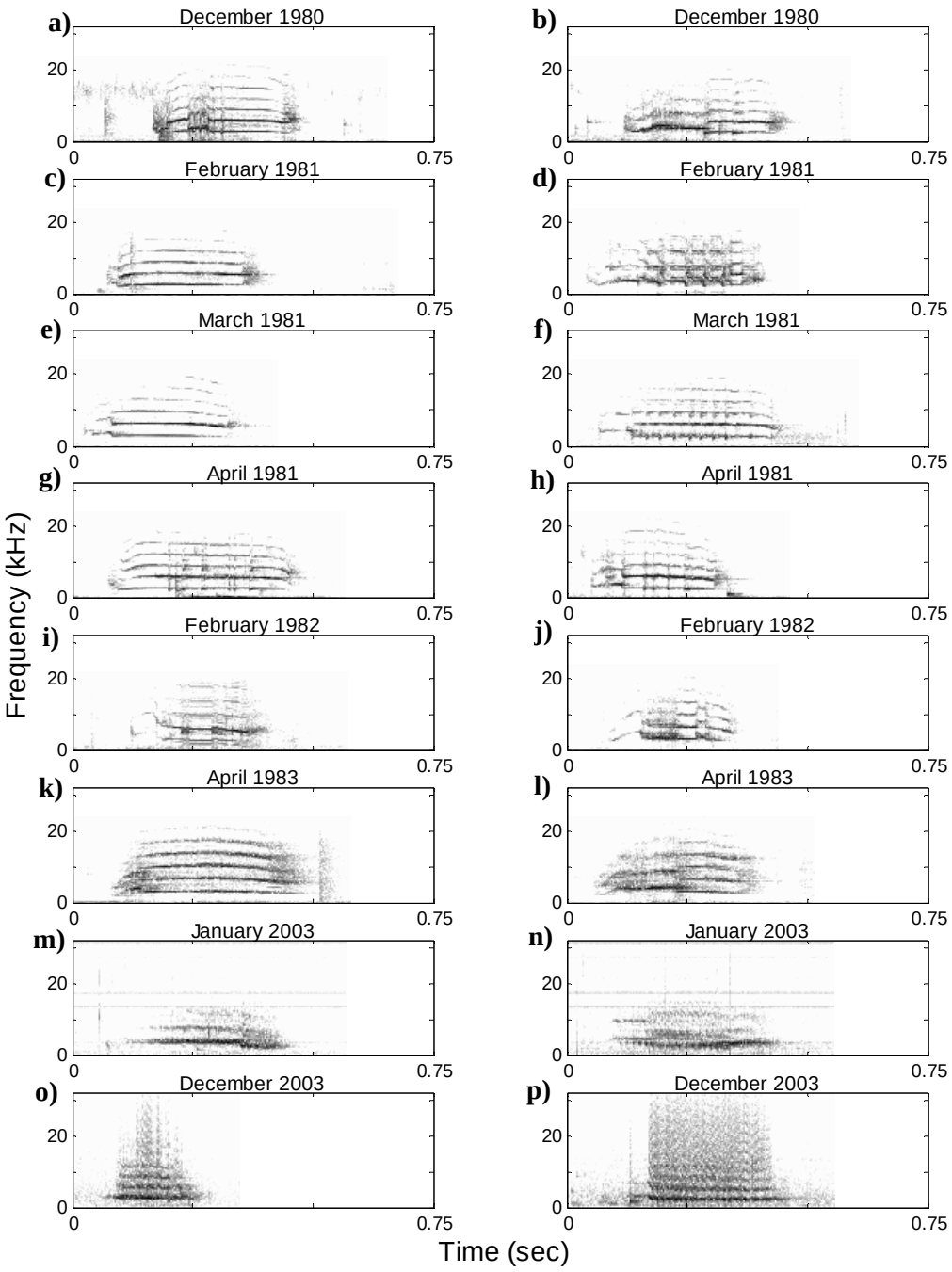

Figure 21. Spectrograms of two examples of Lucille's vocalizations from every month she was recorded, showing variability in her vocalizations.

frequency was not significantly different between calves and adults (Table 9), it is possible that individual manatees change different parameters over time while keeping others stable, as do individual Wied's black tufted-ear marmosets (Jorgensen and French 1998). It is also possible that male and female manatees differ in which parameters they change over time. Such a sex difference was seen in collared doves, where females showed stability in their perch-coos over time whereas males changed several acoustic parameters between the ages of two and 18 months (Ballintijn and ten Cate 1997). Such changes could have contributed to the overall difference in vocalizations of male and female manatee calves found in this study (adult manatees were not analyzed due to the low number of adult males recorded). The percentages of vocalizations correctly classified decreased for Gene and Robin (both males) when the 1980's data were added, but so did Lucille's (a female; Tables 2-3). Thus, a larger sample size is necessary to address the issue of possible sex differences in call stability.

Wilcoxon signed-rank tests performed on the vocal parameters of Gene, the only manatee that was recorded as an adult over a long time period (between 1980 and 2002), indicated that the individual parameters of adult calls also may not be stable over long time periods (Table 6). This high percentage (66.7%) of significant changes concurs with the findings from the CVLDAs, where the percentage of Gene's calls correctly assigned decreased when data from the 1980's were added to the more recent data (Tables 2 and 3). However, due to the small sample size, it is impossible to draw any conclusions on long-term stability of adult vocalizations, and thus future research should address this issue.

During Ontogeny

The second category of ANOVAs focused on manatees that were recorded when they were less than one year old and at least once more when they were between one and three years old (Janice, Lucille, Macon, and Robin). Three out of four of these manatees had low percent changes in their parameters (0-20%), whereas the fourth (Janice) had four of five parameters (80%) significantly decrease over two different field seasons (from when she was a calf to when she was a subadult; Table 7). Three of these parameters (emphasized band, interval 0-1, and interval 3-4) were also significantly different between calves and adults, with calves having significantly higher frequency parameters than adults (Table 9). Thus, as mentioned previously, it is possible that subadults and adults produce vocalizations similar in quality. The large percentage of significant changes seen for Janice (80%) concurs with the results from the CVLDAs, in which a low percentage of her calls was correctly classified when data from both field seasons were used, but much higher percentages were correctly classified when data from a single field season were used, suggesting there may be a stage between being a calf and a subadult in which vocalizations change. The other three calves showed more stability in vocal parameters, although several parameters could not be analyzed because data were not normal and could not be transformed (Table 7). Lucille showed only an increase in fundamental frequency, and no parameters changed in either Macon's or Robin's vocalizations. Thus, no obvious pattern of changes in individual parameters can be seen for calves, especially with such a small sample size. It is possible that there is individual variation in which parameters change over time, if any. It is also interesting to note that Janice, who came in suffering from mild cold stress, was never found to associate with her mother during the second year of recording (personal communication with BSSP Ranger Wayne Hartley 2005). Thus, as a subadult, Janice would not have needed to

communicate with her mother that year like the other three manatees did (as calves), and this potentially could have contributed to the changes that occurred in her vocalizations. It is also possible that the timing of these changes varies among individuals, as suggested previously. The exact ages (to the month) of calves in this study were not known; it was only known when they first appeared with their mother at Blue Spring State Park as a first year calf. Thus, perhaps Janice was undergoing maturational changes in her vocalizations whereas the others had not yet begun to make such changes. Such maturational changes would support the results of the CVLDA on age classes, where the calls of adults and calves were found to be different. Future research should closely monitor vocalizations during the first few years of life in order to examine possible individual differences in vocal ontogeny.

During Adulthood

The third category of ANOVAs focused on animals recorded when they were adults in at least two separate field seasons over a short (1-3 year) time period (Judith, June, Lucille, and Luna—all females). Donna also fit into this category, but due to the small number of vocalizations recorded for her during the second field season, she could not be used for these analyses. Results for these individuals were highly variable, with two showing no changes at all (June and Luna), and two showing between 37-43% significant changes (Lucille and Judith; Table 8). Lucille and Judith showed differences in which parameters they changed as well as differences in how they changed them. The significant decrease in the frequency range of Lucille's vocalizations (which can be seen in the contours of her vocalizations in Figure 21) supports the findings of Sousa-Lima *et al.* (2002), who found that frequency range narrowed over time in Amazonian manatees. However, the frequency range of Judith's vocalizations

significantly increased over two field seasons. Sousa-Lima *et al.* (2002) measured the frequency range of the fundamental frequency, whereas this study measured the emphasized band's frequency range; however, for all of Judith's 31 vocalizations and 79 of Lucille's 82 vocalizations, the emphasized band was the fundamental frequency. As stated previously, the increased duration of Judith's calls concurs with the findings of Sousa-Lima *et al.* (2002), who found that Amazonian manatees increase the duration of their calls with age. While Judith's and Lucille's percent changes may appear high, their percent correct classification rates in the CVLDA was very high when using calls from only their adult years. Judith had 71.0% of her calls correctly classified when using all of the data (Table 3), and Lucille had 68.8% of her calls correctly classified when only using the most recent data (the years she was an adult; Table 2). Thus, although certain parameters of calls may change over short time periods, these changes may not affect their individual distinctiveness. In fact, this also applies to Donna from the first category, who showed 40% significant changes in her parameters. Despite these changes, her correct classification percentage actually increased when using all of the data, which seems to indicate that the changes she made in her call parameters did not affect the individual distinctiveness of her call. Similarly, common marmosets significantly changed some parameters in their phee calls over a year's time while still maintaining very high levels of individual distinctiveness (Jones *et al*. 1993). In addition, Jorgensen and French (1998) found that some Wied's black tufted-ear marmosets changed only a few parameters of their phee calls over three years whereas others changed many parameters. Like what I found in adult manatees, these marmosets did not show similar changes in parameters either, and yet they still maintained high levels of individual distinctiveness in their phee calls during each individual year

(Jorgensen and French 1998). Once again, this same idea could also apply to those individuals recorded over 19 years.

Overall, findings from this study suggest that many call parameters are not stable from calf to adulthood in at least some individuals. In addition, some calves, subadults, and adults show variable changes in parameters over short time periods whereas others show stability in all parameters over short time periods. Thus, the results from this study do not support the hypothesis that individual Florida manatee vocalizations are stable over long time periods, but future research still needs to be done on this topic due to small sample size and the use of only one adult. Questions still remain about the hypothesis that calls are stable over short time periods, again due to small sample size, as well as to the amount of individual variability in the number and type of parameters that changed.

This study indicates that both calves and adults can change certain parameters over short time periods and that these parameters can vary for different individuals. While certain parameters may not be stable over short or long time periods, other parameters (or combinations of parameters) that could be important in determining individual variability, including ones not addressed in this study, may remain stable. It is also possible that individuals have different parameters that are important for determining individual distinctiveness. Since the vocalizations of most animals were classified correctly in the CVLDA, it appears that at least some of the parameters that I measured are important to individual distinctiveness of vocalizations. However, others may also be important, such as contour (the pattern of frequency changes over time), which has been found to be stable in Amazonian manatees for up to four years (Sousa-Lima *et al.* 2002). Future work on vocal stability should include parameters found to be

important in previous studies, such as contour, all subharmonics, minimum fundamental frequency, maximum fundamental frequency, and the center frequency of the first band.

Although many differences between the vocalizations of calves and adults are likely due to maturation, possibly occurring during the subadult stage, there are other possible factors that can influence such changes. These include social influences, hormonal fluxes not due to maturation, motivational state, and context. As mentioned previously, prolonged exposure to conspecific calls or the introduction of new conspecifics have been found to influence call structure in several species (Jorgensen and French 1998). Production learning, in which animals learn to produce sounds based on auditory input, can cause an animal to make either similar or dissimilar vocalizations to the ones to which it is exposed (Janik and Slater 2000). Any of these factors are likely to be different in captivity than in the wild, and thus may have contributed to the misclassifications across age classes seen in the vocalizations of Georgia and Lorelei, who may have experienced increased and prolonged exposure to certain age classes that manatees may not normally experience in the wild. Possible effects of social influences or learning on manatee calls are areas in need of exploration.

Because male manatees cease spermatogenic activity in winter (Hernandez *et al.* 1995) and because mature females have multiple one-month estrous cycles a year (Reynolds and Odell 1991), these hormonal fluctuations could cause changes in vocal parameters. A one-year study on mature captive manatees could provide insight into whether or not differences in hormonal levels cause vocal parameter changes.

As mentioned previously, motivational state and context could also be reasons for intra-individual variation in manatee call parameters. Sousa-Lima *et al.* (2002) suggested that the intra-individual variation found in the duration of Amazonian manatee calls might be due to

different arousal states. Whether or not manatee vocalizations are context-specific is yet to be determined. If manatees do have context-specific calls, it is possible that only one call type has individually distinctive features, such as the isolation calls of many species. Contact calls (i.e., between mothers and calves) seem to represent a large proportion of manatee calls; if these were the only calls that are individually distinctive, this could contribute to the lower percentages of calls correctly classified for some individuals. Future research needs to explore the possibility that manatees produce context-specific calls, and to examine the individual variability of each call type, should different call types occur.

Behaviors and Vocalizations

Group size did not affect vocalization rates for manatees at Blue Spring and the adjoining St. Johns River, which contrasts with the findings of Reynolds (1981) and Alicea-Pou (2001) that groups of more than two animals vocalized more than smaller groups (one or two, excluding mother-calf pairs). I did not, however, separate groups with mother-calf pairs. Manatees vocalized during all behaviors at different rates, thus supporting the hypothesis that Florida manatee vocalization rates vary depending on behavior (Table 10). When looking at calls per minute per manatee, manatees that were playing had significantly lower vocalization rates than those participating in any other behavior (Tables 10 and 11), possibly because play was the only behavior that was purely solitary. Manatees that were bottom resting also were found to have low vocalization rates, significantly lower than manatees that were milling, socializing, surface resting, and traveling (Tables 10 and 11). Lower call rates during bottom resting have also been found in previous studies (Bengtson and Fitzgerald 1985, Alicea-Pou 2001). Finally, manatees that were surface resting had significantly higher vocalization rates than those that were feeding

(Table 11). When looking at calls per minute averaged across group sizes, social activity resulted in high vocalization rates, again significantly higher than bottom rest (Tables 10 and 12). Socializing manatees also had higher vocalization rates than those that were playing or with boat (Table 12). Mill and surface rest resulted in significantly higher vocalization rates than bottom rest, and mill resulted in higher vocalization rates than with boat (Table 12). The inclusion of nursing in my definition of feeding may have affected the feeding rates, although this occurred very rarely in my observations that I included in these analyses (approximately 6.7% of the manatee minutes in the feeding category). I also did not examine the possibilities that there are context-specific call types or that manatees may change certain parameters of their calls during specific behaviors; these are areas that need to be examined in the future.

I conducted most of my fieldwork in a canoe, which may have affected manatee vocalization rates during all behaviors. However, I tied up the canoe by the side of a bank for most observations and started making observations several minutes after I had approached a group. Although Bengtson and Fitzgerald (1985) found that manatees stopped vocalizing in the presence of canoes, Alicea-Pou (2001) reported that they began vocalizing again soon afterward (while still in the presence of a canoe).

I often heard mothers vocalize to their calves when changing behaviors (i.e., from bottom resting to milling/traveling); other researchers have also found that mothers and calves vocalize to maintain contact (Hartman 1979, Steel 1982, Bengtson and Fitzgerald 1985, Ripple 1999, O'Shea *et al.* 2000). Another interesting observation I made had to do with the manatees' behavior toward armored catfish (*Pteryogoplichthys disjunctivus*, new to the Park during the 2002-2003 field season after being introduced from someone's aquarium upstream; personal communication with Rangers at BSSP 2002). These catfish were found to eat algae off the

manatees' skin, and as many as 46 were counted on a single manatee. The rangers thought that the catfish would sometimes bite off a piece of the manatees' skin, which would cause them to squirm and roll (an increase in both of these activities were seen during 2002-2003; personal communication with BSSP rangers 2002). I also noticed an increase in manatees with fish attached switching from bottom resting to milling, and that manatees usually vocalized when beginning to squirm and roll.

In summary, the hypothesis that Florida manatees have individually distinctive vocalizations has been supported by this study, with a CVLDA using nine parameters from the vocalizations of 33 manatees. The hypothesis that individual Florida manatee vocalizations remain stable over long time periods was not supported, either by the CVLDAs or by ANOVAs/Wilcoxon signed-rank tests on vocal parameters of individuals recorded over 19+ years. However, stability in adults has only been examined in one individual. Conflicting evidence was found for short-term stability in both calf and adult calls, with the number and type of parameters that changed being highly variable. CVLDAs and ANOVAs indicated that differences exist between the calls of calves and adults, which could possibly be due to maturation. A CVLDA also found differences in the vocalizations of male and female calves. For manatees at Blue Spring and the adjoining St. Johns River, group size did not affect vocalization rates of manatees engaged in different behaviors. Behaviors did affect vocalization rates, with the highest rates occurring during social activity and the lowest rates occurring during bottom resting.

This study adds to a growing body of data indicating that manatee calls are communicative in nature, individually distinctive, possibly stable over short time periods, and

produced at different rates during different behavioral states. Playback experiments need to be carried out to determine if calls are used in individual recognition. Further study of individual recognition and vocal stability could provide insights into manatee social structure. For example, if calls are used for individual recognition and show only short-term stability, then their primary function might be to facilitate contact between mothers and their calves, which typically associate together for only a few (1-2) years but remain in contact as adolescents (subadults) as well (Reynolds and Odell 1991). Short-term stability could also facilitate other short-term relationships, such as those occurring during aggregation at a winter refuge. On the other hand, if calls are stable over long time periods, then individual recognition could facilitate long-term, individually specific social relationships, especially if manatees live in fission-fusion societies as suggested by Reynolds and Powell (2002). Results of this study also indicate that acoustic monitoring could be used to document the presence of specific individuals, the presence of manatees from different age classes and sexes, or the occurrence of certain activities (such as social or bottom rest) without having to conduct boat-based surveys, thus potentially contributing to manatee conservation efforts.

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APPENDIX

Appendix A: Manatee Behaviors (based on definitions described by Koelsch (1997))

Surface Rest—floating or basking at the surface; no change in location

Bottom Rest—low level of activity at the bottom of the water column, no change in location, and surfacings at regularly spaced intervals for breaths

Mill—moving without directionality or rolling and squirming due to fish

Travel—directed movement

Social—two or more manatees interacting (including nudging, bumping, kissing, and any mating-like behavior); this category includes cavorting, which has been defined by others as vigorous interactions between manatees such as pushing, nuzzling, rolling, mouthing, embracing, and other mating-like behavior (Bengtson and Fitzgerald 1985, Koelsch 1997)

Feed—visible food in a manatee's mouth, and/or chewing, nursing, or grazing (defined as mouth touching plants)

With boat—manatee approaches my canoe and touches, rubs, or stays within two feet of canoe

Play—interacting with a non-food object such as a piling or line